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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: October 06, 2011

SUBJECT: Aminocyclopyrachlor and aminocyclopyrachlor-methyl DERs

PC Codes: 288008, 288009
Decision Nos.: 443546

Petition No.: None
Risk Assessment Type: Tox DERs
TXR No: 0056049
MRID Nos: (see below)

DP Barcodes: D386645
ID No.: 352-782-Dupont Aminocyclopyrachlor Technical
Regulatory Action: First Food Use (R150)
Case No.:
CAS No.: 858956-08-08, 858954-83-3
40 CFR: NA

Ver.Apr.08

FROM: Jessica Ryman, Ph.D., D.A.B.T., Toxicologist 10/6/2011
Abdallah Khasawinah, Ph.D., Toxicologist 10-6-2011
Risk Assessment Branch IV
Health Effects Division, 7509P

THROUGH: Felicia Fort, Acting Chief 10/6/2011
Risk Assessment Branch IV
Health Effects Division, 7509P

TO: Mindy Ondish
Herbicide Branch
Registration Division (7505P)

I. CONCLUSIONS

The DERs supporting the first food use for aminocyclopyrachlor and aminocyclopyrachlor-methyl have been reviewed by HED. All have been classified as acceptable/guideline.

II. ACTION REQUESTED

None.

Recd in RM 10/12/11

1 of 88

III. BACKGROUND

Non-food uses of the herbicide aminocyclopyrachlor were registered in 2010. The registrant has now submitted studies to support the first food use of aminocyclopyrachlor. These studies are summarized in the MRID Summary Table (below).

IV. ATTACHMENTS: SUMMARY TABLE

Guideline	MRID	Comments	File Name
870.4200b	48333606	New DER	48333606.DER
870.4300	48333607	New DER	48333607.DER
870.4100	48333608	New DER	48333608.DER
870.7485	48333609	New DER	48333609.DER
870.7485	48333610	New DER	48333610.DER

DATA EVALUATION RECORD

^{14}C-Aminocyclopyrachlor (DPX-MAT28)
PC Code: 288008
TXR#: NA
MRID#: 48333606

Combined Chronic Toxicity/Carcinogenicity Study - Mice
OPPTS 870-7485

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Principal Reviewer _____ Date 5-19-11
Daniel Ewald

Secondary Reviewer _____ Date 5/20/2011
Henry Spencer, Ph.D.

Tetrahedron Program Manager Nasrin Begum Date 5/23/11
Nasrin Begum, Ph.D.

Quality Control Nasrin Begum Date 5/23/11
Nasrin Begum, Ph.D.

Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-42
EPA Reviewer/WAM: Ryman/Ottley

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Ryman, Jessica, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10/6/2011
EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10-6-2011
Work Assignment Manager: Lori Brunsman **Signature:**
Science and Information Management Branch, Health Effects Division (7509P) **Date:**
Template version 02/06

TXR#: 0056049

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity 18-Month Feeding Study in Mice, OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 288008 **DP BARCODE:** D386645

TEST MATERIAL (PURITY): Aminocyclopyrachlor (DPX-MAT28) (88.3 - 90.5%)

SYNONYMS: Aminocyclopyrachlor (DPX-MAT28)

CITATION: Jung-Im Huh, Ph.D. (2010). DPX-MAT28 Technical: Carcinogenicity 18-Month Feeding Study in Mice. Korea Institute of Toxicology, Republic of Korea. Laboratory Study Number: IG07280, October 15, 2010 MRID 48333606. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company Wilmington, Delaware 19898.

EXECUTIVE SUMMARY:

The objective of this study (MRID 48333606) was to assess the carcinogenic potential of DPX-MAT28 Technical (DPX-MAT28) in mice. Five groups of young adult male and female Crlj:CDl(ICR) mice (60/sex/group) were administered diets that contained 0, 300, 1000, 3000, or 7000 ppm (corresponding to 0/0, 39/50, 133/171, 393/527, 876/1190 mg/kg bw/day) DPX-MAT28 for approximately 18 months.

Body weights and food consumption were evaluated weekly for the first 13 weeks, then every other week thereafter. Detailed clinical observations were evaluated weekly. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. White blood cell differential counts (via blood smear) were evaluated in surviving mice at the end of the exposure period and in mice that were sacrificed in extremis. After approximately 18 months of dietary exposure, mice were sacrificed and given a gross and microscopic pathological examination.

No test article-related changes were observed in the following observations in male and female mice fed up to 7000 ppm DPX-MAT28: clinical observation, body weight parameters, food

intake parameters, ophthalmology, white blood cell differential counts, cause of death, organ weights and gross pathological parameters, and neoplastic or non-neoplastic changes.

DPX-MAT28 is not an oncogen in mice.

The NOAEL for chronic toxicity is 7000 ppm (876/1190 mg/kg bw/day in males and females). The LOAEL was not observed.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a carcinogenicity study (OPPTS 870.4200b) in mice.

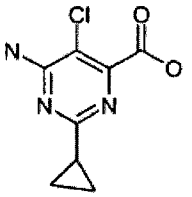
COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality Claim statements were provided.

A flagging statement was also included noting that this study does not meet or exceed criteria in EPA 40 CFR part 158.34.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

Description:	DPX-MAT28 technical
	White powder
Lot/batch #:	DPX-MAT28-023
Purity:	90.5% per COA revision 1 on 8/30/2007 88.3% per COA revision 2 on 12/3/2008
Compound stability:	Stability information for the test substance was documented in the Certificate of Analysis which designated the expiration date for the batch of test substance used in this study as May 21, 2010.
CAS # of TGAI:	CAS # 858956-08-8
Structure:	

2. Vehicle and/or positive control: Not applicable – DPX-MAT28 mixed directly into food

3. Test animals:

Species:	Mice - male and female
Strain:	Crlj:CD1(ICR)
Age/weight at study initiation:	Approximately 4 weeks old; Weight range for males: 27.5-38.4 g. Weight range for females: 20.7-28.1 g.
Source:	Charles River Laboratories, Japan
Housing:	Animals were housed individually in shoe bottom cages before and during the study.
Diet:	Lab Diet® #5002, PMI Nutrition International, USA; (irradiated by gamma-ray) *ad libitum*
Water:	Tap water, filtered and irradiated by UV light (municipal water supply)*; ad libitum.*
Environmental conditions:	**Temperature:** 23±3 °C **Humidity:** 40-60% **Air changes:** Not described **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	6 days.

B. STUDY DESIGN:

1. In life dates: April 18, 2008 (In-Life Start)
October 29, 2009 (In-Life Termination)

2. Animal assignment: Animals (300/sex) were assigned to treatment groups as noted in Table 1. Mice were randomized and assigned to treatment groups based on body weight. The weight variation was within ±20% of the mean for each sex.

Table 1 – Study Design

Test Group	Conc. in diet (ppm)	# Male	# Female
Group 1 – Control	0	60	60
Group 2	300	60	60
Group 3	1000	60	60
Group 4	3000	60	60
Group 5	7000	60	60

3. **Dose selection rationale:** Doses were selected by the Sponsor based on the results of a sub chronic study. The 7000 ppm level was expected to produce non-lethal effects. The 300 ppm level was expected to be a no observed-adverse-effect level (NOAEL). The intermediate concentrations were expected to produce a dose-response for any observed effects. The 7000 ppm level was expected to deliver the limit dose (1,000 mg/kg/day) dictated by international regulatory test guidelines for the carcinogenicity study.

4. **Diet preparation and analysis:** DPX-MAT28 was mixed with diet using a V-type mixer. The diets were formulated biweekly and stored in the refrigerator [approximately 4°C, Archives No.: KIT-401(48), Archives No.: KIT-401(51)] until it was given to the animals.

   Samples were analyzed to verify concentration, homogeneity, and stability of DPX-MAT28 in the diets. Diet samples were collected from all concentrations every 3 months to verify concentration. All remaining samples were discarded after satisfactory analytical results.

   Duplicate samples of all dietary concentrations were collected at the initial diet preparation and analyzed to verify the concentration (average of homogeneity samples) and homogeneity of DPX-MAT28 in the diets. DPX-MAT28 in diet was shown to be stable at concentrations of 600 and 18000 ppm in the 2-year rat feeding study (DuPont-22790). Since 7000 ppm concentration was within the range of 600 to 18000 ppm, stability test was performed only for 300 ppm in this study.

5. **Results:**

   Homogeneity analysis: The homogeneity results show that the test article was mixed homogeneously (CV's = 0.5-3.8, 0.8-1.2, 0.7-4.9 and 1.5-1.9% for 300, 1000, 3000, and 7000 ppm samples, respectively) and at the targeted concentrations (± 8.1% of nominal) for all dietary levels.

   Stability analysis: The stability of the diets at concentrations of DPX-MAT28 is as follows:

Sample Type	300 ppm (% Nominal)
0 Day Room Temperature[a]	100.0
0 Day Room Temperature	90.0
0 Day Room Temperature	89.2
14 Day Refrigerated	97.9
14 Day Refrigerated	100.4

a - The 0-day room temperature result is the mean result from the top, middle, and bottom homogeneity sample analysis. The homogeneity samples were frozen immediately upon diet preparation and used as baselines for comparison with the stability results.

   Concentration analysis: The concentration verification results show that the test article was at the targeted concentrations (+ 8.5% of nominal) for all dietary levels.

6. **Statistics:** Statistical analyses were performed by comparing the different dose groups with the vehicle control group using Path/Tox System (ver. 4.2.2, Xybion Medical

Systems Corporation, USA) and/or Statistical Analysis Systems (SAS/STAT Version 9.1.3. or 9.2, Gary, NC, USA). Statistical analyses for survival and microscopic findings were performed by the Sponsor. Statistical Analyses were carried out as follows:

		Method of Statistical Analysis	
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain Food Consumption Food Efficiency Clinical Pathology Incidence of Ophthalmology Observations Organ Weight	Bartlett's test for Homogeneity[a]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's Rank Sum test
Survival	None	Cochran-Armitage test for trend[b,c] Kaplan-Meier analysis	
Incidence of Microscopic Lesions	None	Cochran-Armitage test for trend[b,d,e] Fisher's exact test	

a - When the Bartlett test indicated no significant deviations from variance homogeneity, the ANOVA multiple comparison test (and Dunnett's test, if necessary) was conducted to determine which pairs of group comparison were significantly different. Significant deviations from variance homogeneity were observed, a non-parametric comparison (Kruskal-Wallis H Test) was conducted. When a significant difference was observed in the Kruskal-Wallis H Test, the Dunn's Rank Sum Test was conducted to determine the specific pairs of group comparison which were significantly different.
b - If the incidence was not significant, but a significant lack of fit occurred, then Fisher's exact test with a Bonferroni-Holm correction was used.
c - The trend test was applied to the data sequentially. If a significant dose-response was detected, data from the top dose group was excluded and the test repeated until no significant trend was detected.
d - If tissues (subject to availability) were not evaluated microscopically in all groups, or if it was unlikely that most tumors were observed grossly, then Fisher's exact test was used.
e - Cochran-Armitage test for trend was applied sequentially to evaluate microscopic observations if all groups were evaluated. Otherwise, Fisher's Exact test was used with a Bonferroni-Holm correction

C. METHODS:

1. Observations:

1a. Cageside observations: Cage-side examinations to detect moribund or dead mice (including spares during the first two weeks) were conducted once daily in the morning throughout the study except during the acclimation period. The animals were observed more than once when they were in poor condition.

1b. Clinical examinations: Additional cage-site evaluations to detect acute clinical signs of systemic toxicity, abnormal behavior and/or appearance among mice were conducted once a day, except on the days when detailed clinical observations were conducted. Additional observations were performed to monitor animals considered to be in poor condition more closely.

Detailed clinical examinations were conducted during the pre-treatment period (including spare animal), at the first day of dosing prior to test article exposure and once a week thereafter.These examinations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic tonic, stereotypical, or bizarre behavior and presence of masses etc. Any abnormal clinical sign noted was recorded weekly up to Week 77 for all animals.

Detailed clinical observations were not performed on Weeks 78 and 79 in error prior to

necropsy for all animals. Since animals were observed daily at least twice, not performing detailed clinical observation does not impact the study. These are considered not to affect the integrity of the study.

1c. **Ophthalmic Exams:** Ophthalmological examinations were conducted by three veterinarians with competency in ophthalmological evaluation designated by KIT Management Director. Ophthalmological examination on all animals was conducted once during the pre-treatment period (including spare animals) and at the end of study. All surviving mice were examined prior to the final sacrifice. Both eyes of each mouse were examined by focal illumination and indirect ophthalmoscope (IOH, Neitz Instrument Co., Japan) after mydriasis had been produced with Mydrin-P (Santen Pharmaceutical Co., Japan).

2. **Body weight:** Body weight was measured twice during acclimation period (Days -14 and -9) and once during pre-treatment period (day -4). During the treatment period, all animals were weighed on Day 0 prior to test article exposure, at the first day of every study week during the first 13 weeks, every other week thereafter and stopped at the last biweekly prior to necropsy. On the necropsy day, animals were weighed prior to necropsy.

3. **Food consumption and compound intake:** Individual food consumption of all animals was calculated during pre-treatment (Week -1) and weekly during the first 13 weeks and every other week thereafter. The amount of food consumed by each mouse over each weighing interval was determined by weighing each feeder at the beginning and end of the interval and subtracting the final weight and dividing by the number of animals in the cage. From these measurements, mean daily food consumption over the interval was determined. The mean daily food efficiency and mean daily intake of test article were calculated using food consumption and body weight.

4. **Hematology:** The study report stated that blood smears were prepared for evaluation of differential WBC counts from all mice sacrificed in extremis and from all mice at 12 and 18 months. Differential WBC counts were evaluated in all mice sacrificed in extremis and in control and high-concentration mice from the necropsy. It was stated in the study report that results were reported only for mice sacrificed in extremis and in control and high concentration mice from necropsy. Approximately 0.2 ml/mouse blood was collected from lateral tail vein of each animal at 12 months and from the abdominal vena cava of each animal, while the animal is under isoflurane anesthesia at 18 months necropsy without fasting. Blood smears were prepared at the time of blood collection and were stained with Wright-Giemsa stain for microscopic evaluation.

5. **Sacrifice and pathology**:
Complete necropsy examinations were performed under the direct supervision of veterinary pathologists on all animals found dead (tissue integrity permitting), accidentally killed, euthanized in extremis, and euthanized at scheduled necropsy (test days 546 to 552 for males and 556 to560 for females, respectively). No necropsy was performed on spare animals. Animals were euthanized by isoflurane inhalation. After blood sampling, the animals were killed by exsanguination from the vena cava and aorta.

The following tissues were collected from mice. Paired organs were weighed together, and any weight for an organ with a mass was noted.

Digestive System Liver/gall bladder[a] esophagus stomach duodenum jejunum ileum cecum colon rectum salivary glands pancreas	Reproductive System Male Testes[a] Epididymides[a] prostate seminal vesicles Female ovaries (with oviducts)[a,d] uterus (including cervix) [a,d] mammary glands vagina	Hematopoietic System spleen[a] thymus mandibular lymph node mesenteric lymph node bone marrow[b] Peyer's patches[c] Nervous System Brain[a] (including cerebrum, cerebellum, medulla/pons) eyes (including retina and optic nerve) spinal cord (cervical, mid-thoracic, lumbar) sciatic nerve
Musculoskeletal System skeletal muscle femur/knee joint[b] sternum	Endocrine System pituitary gland thyroid gland[a] parathyroid glands adrenal glands	Respiratory System lungs trachea nose larynx pharynx
Urinary System Kidneys[a] urinary bladder	Cardiovascular System Heart[a] aorta	Miscellaneous Skin Eyes (including retina and optic nerve) Gross observations[e]

a. Organs were weighed at necropsy.
b. Bone marrow was collected with the femur and sternum. Bone marrow smears were stained with Wright-Giemsa stain, but analysis was not necessary to support experimental findings.
c. Peyer's patches were collected with sections of digestive tract.
d. Uterus was weighed with cervix. Ovaries were weighed with oviducts.
e. Gross observations made at necropsy for which histopathology was not appropriate (e.g., fluid, ruffled fur, and missing anatomic parts) were not collected

Preservation of Tissues: All tissues from each animal were preserved in 10% neutral buffered formalin with the following exceptions: the eyes (including retina and optic nerve) were fixed in Davidson's fixative; and the testes and epididymides were fixed in Bouin's fixative for approximately 24 hours, and then transferred to 70% ethanol. Formalin was infused into the lung via the trachea and into the urinary bladder.

6. **Histopathology:** All tissues collected from mice at the 18-month time point, in the high-concentration and control groups, and from mice that were found dead or accidentally killed (tissue integrity permitting), or were sacrificed in extremis, were further processed to slides, stained with hematoxylin and eosin, and examined microscopically. Gross lesions and liver at 300, 1000, and 3000 ppm were evaluated microscopically. Selected gross observations for which microscopic diagnosis were not additive to the interpretation of the study were saved, but were not processed for microscopic evaluation.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no test article-related clinical signs observed in this study.

2. **Mortality:** There was no effect of the test article on mortality.

B. BODY WEIGHT AND WEIGHT GAIN:

There were no test article-related effects on body weights or body weight gains in males or females (Table 3). Statistically significant changes observed were not considered test article-related as they were sporadic in nature, resolved by the end of the study, and did not impact overall body weight over the duration of the study.

Similarly, no test article effects on body weight gain were observed in either sex.

TABLE 3: Mean bodyweights (BW) and bodyweight gains (BWG)[a]

g ± SD	0	300 ppm	1000 ppm	3000 ppm	7000 ppm
MALES Initial BW	33.6 ± 1.86	33.5 ± 1.99	33.2 ± 1.88	33.2 ± 2.10	33.7 ± 1.72
Day 21	37.3 ± 2.04	37.9 ± 2.43	37.2 ± 2.29	36.7 ± 2.70	36.2 ± 2.16* (↓2.9%)
Day 42	40.4 ± 2.27	40.4 ± 2.84	39.9 ± 2.47	38.7 ± 3.05	39.0 ± 2.62* (↓3.5%)
Day 49	40.9 ± 2.39	40.9 ± 3.02	41.2 ± 2.61	38.7 ± 3.11** (↓5.4%)	39.5 ± 2.69* (↓3.4%)
Day 539	51.2 ± 5.88	52.2 ± 6.52	51.1 ± 5.09	49.1 ± 5.17** (↓4.1%)	52.0 ± 5.92* (↑1.5%)
Overall BWG Wk -1-75	17.4 ± 5.83	18.7 ± 5.46	18.1 ± 5.11	15.9 ± 4.28	18.2 ± 5.55
FEMALES Initial BW	24.3 ± 1.21	24.3 ± 1.42	24.5 ± 1.23	24.8 ± 1.22	24.5 ± 1.21
Day 21	27.3 ± 1.37	27.5 ± 2.05	27.6 ± 1.86	27.8 ± 1.88	28.2 ± 1.83
Day 42	29.8 ± 2.11	29.6 ± 2.55	30.1 ± 2.54	29.1 ± 2.24	29.6 ± 2.07
Day 49	29.5 ± 1.93	30.2 ± 2.52	30.4 ± 2.75	30.0 ± 2.53	29.7 ± 2.32
Day 539	40.7 ± 5.22	43.1 ± 8.07	41.7 ± 8.50	41.3 ± 6.08	41.2 ± 7.36
Overall BWG Wk -1-75	16.5 ± 5.28	18.8 ± 7.50	17.5 ± 8.20	16.4 ± 5.70	16.6 ± 7.08

*p<0.05 compared to control; **p<0.01 compared to control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:**
No test article-related effects on food consumption and food efficiency were observed in male or female mice. Some statistically significant changes were observed, but these were considered sporadic as they resolved prior to the end of the study and did not impact overall food consumption over the duration of the study.

2. **Compound intake:** Information from table 6 was obtained from pages 105 and 111 of the study.

Table 4 – Average Daily Intake of DPX-MAT28 (mg/kg/day)					
Sex	**Stat**	**300 ppm**	**1000 ppm**	**3000 ppm**	**7000 ppm**
Male	Mean	3.87	132.7	393.1	876.2
	S.D.	4.19	13.19	50.28	85.16
Female	Mean	49.9	170.6	526.8	1190.0
	S.D.	7.33	24.43	58.03	147.33

D. OPHTHALMOSCOPIC EXAMINATION: There were no test article related changes. All observations were considered within normal limits for mice of this strain and age.

E. BLOOD ANALYSES:

1. **Hematology:** There were no test article-related changes in blood smear evaluations in males or females.

2. **Clinical chemistry:** Not conducted

F. URINALYSIS: Not conducted

G. SACRIFICE AND PATHOLOGY:

1. **Necropsy:** The causes of death in all treatment groups were comparable to those of control group and were typical of aging Crlj:CDl(ICR) mice in a chronic feeding study.

   In males and females 18-month survival ranged from 45% to 49% and 39% to 46% respectively. The most common cause of early death in both sexes was malignant lymphoma, which was not considered test article-related.

2. **Organ Weights:** No differences in absolute or relative organ weights were observed in either sex.

3. **Histopathology**

   Gross Findings: There were no test article-related gross changes in either sex. The incidences of all gross changes in all treated groups were consistent with normal background lesions in the mice of this age and strain.

   Microscopic Findings: Daily dietary exposure of male and female mice up to 7000 ppm of the test article, for approximately 18 months, was not oncogenic in male or female mice. There were no test article related non-neoplastic microscopic findings in male or female mice.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

DPX-MAT28 is not an oncogen in mice. Under the conditions of this study, the no-observed adverse-effect level (NOAEL) for DPX-MAT28 was considered to be 7000 ppm (the highest concentration tested) for male and female mice. This concentration was equivalent to 876 and 1190 mg/kg/day in males and females, respectively.

The NOAEL is based on a lack of adverse test article-related effects on any in-life parameter, white blood cell differential counts, or anatomic pathology parameters in male and female fed up to 7000 ppm.

B. REVIEWER COMMENTS:

The objective of this study (MRID 48333606) was to assess the carcinogenic potential of DPX-MAT28 Technical (DPX-MAT28) in mice. Five groups of young adult male and female Crlj:CDl(ICR) mice (60/sex/group) were administered diets that contained 0, 300, 1000, 3000, or 7000 ppm (corresponding to 0/0, 39/50, 133/171, 393/527, 876/1190 mg/kg bw/day) DPX-MAT28 for approximately 18 months.

Body weights and food consumption were evaluated weekly for the first 13 weeks, then every other week thereafter. Detailed clinical observations were evaluated weekly. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. White blood cell differential counts (via blood smear) were evaluated in surviving mice at the end of the exposure period and in mice that were sacrificed in extremis. After approximately 18 months of dietary exposure, mice were sacrificed and given a gross and microscopic pathological examination.

No test article-related changes were observed in male and female mice fed up to 7000 ppm DPX-MAT28. Clinical observations, body weight parameters, food intake parameters, ophthalmology, white blood cell differential counts, cause of death, organ weights and gross pathological parameters, and neoplastic or non-neoplastic changes were comparable among treated and control groups.

DPX-MAT28 is not an oncogen in mice.

The NOAEL for chronic toxicity and oncogenicity is 7000 ppm (876/1190 mg/kg bw/day in males and females). The LOAEL was not observed.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a carcinogenicity study (OPPTS 870.4200b) in mice.

C. **STUDY DEFICIENCIES** The following deviations/deficiencies were noted that did not affect the integrity or acceptability of the study:

Due to a mistake, no blood smears were prepared from animal number 4F0053 sacrificed in extremis on Day 245. Since there were adequate numbers of blood smears from other animals, omitting this analysis was considered unlikely to affect the integrity of the study.

Detailed clinical observations were not performed on Weeks 78 and 79 in error prior to necropsy for all animals. Since animals were observed daily at least twice, not performing detailed clinical observation does not impact the study. These are considered not to affect the integrity of the study.

Animal numbers 4M0031 and 4M0058 were replaced with spare animals due to loss of tail and hemophthalmia respectively during pretreatment period. Food consumption was not measured for replacement animals during pretest and therefore, no pretest data presented for these animals. Body weight of animal number 2F0052 was entered instead of food quantity on Day 455 so food consumption was not measured on Day 469. These deviations are minor and considered unlikely to affect the integrity of the study.

One found dead spare male and 4 moribund spare males during the pretreatment period were not given a gross examination by mistake. Group-housed males were fighting and the cause of death for these animals were assumed to be fighting and not due to any disease or infection. This deviation was not considered to affect the integrity of the study.

Four males were isolated from the group from Day -6, 2 males from Day -5, and 1 male from Day 3 due to fighting when group housed in a shoe bottom cage.

DATA EVALUATION RECORD
^{14}C-Aminocyclopyrachlor (DPX-MAT28)
PC Code: 288008
TXR#: NA
MRID#: 48333607

Combined Chronic Toxicity/Carcinogenicity Study - Rats
OPPTS 870-7485

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

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Principal Reviewer _____ Date 5-19-11
Daniel Ewald

Secondary Reviewer Henry B. Spencer, Ph.D. Date 5/30/2011
Henry Spencer, Ph.D

Tetrahedron Program Manager Nasrin Begum Date 5/23/11
Nasrin Begum, Ph.D.

Quality Control Nasrin Begum Date 5/23/11
Nasrin Begum, Ph.D.

Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-42
EPA Reviewer/WAM: Ryman/Ottley

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Jessica Ryman, Ph D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10/6/2011
EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10-6-2011
Work Assignment Manager: Lori Brunsman **Signature:** _____
Science and Information Management Branch, Health Effects Division (7509P) **Date:** ____
Template version 02/06

TXR#: 0056049

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity [diet] – [rat]; OPPTS 870.4300 [§83-5]; OECD 453.

PC CODE: 288008, 288009, 288010 **DP BARCODE:** D386645

TEST MATERIAL (PURITY): DPX-MAT28 Technical (Purity: 90.5-88.3%).

SYNONYMS: Aminocyclopyrachlor; 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid, 4-Pyrimidinecarboxylic acid (CAS uninverted); 6-amino-5-chloro-2-cyclopropyl-(CAS inverted); 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid (IUPAC)

CITATION: Moon, Kyoung. (2010). DPX-MAT28 Technical: Carcinogenicity 2-Year Feeding Study in Rats. Korea Institute of Toxicology, Daejeon, Republic of Korea. Laboratory Study Number: IG07280, November 24, 2010, MRID 48333607. Unpublished .

SPONSOR: E.I. du Pont de Nemours and Company Wilmington, Delaware 19898

EXECUTIVE SUMMARY: Five groups of Main male and female Crl:CD(SD) rats (70/sex/group) were administered DPX-MAT28 in the diet at concentrations of 0, 600, 2000, 6000, or 18000 ppm (0/0, 27/29, 279/309, 892/957 mg/kg bw/day in males/females) for 2 years. Satellite animals (10/sex/dose) were administered DPX-MAT28 at these same dietary concentrations. For both Main and Satellite animals, body weights and food consumption were evaluated prior to dietary exposure, weekly for the first 13 weeks, and then biweekly thereafter. Body weights were also recorded on the day of necropsy. Detailed clinical observations were performed prior to dietary exposure, on the day of first dietary exposure, and weekly thereafter. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. For Satellite animals, hematology, clinical chemistry, and urinalysis were performed at 6 months and 1 year and coagulation measurements were performed at 1 year. For Main Study animals, blood smears and white blood differential counts were performed at 1 year and 2 years and for animals sacrificed *in extremis*. Satellite and Main Study animals were sacrificed, organ weights were recorded, and gross and histopathology performed at 1 year and 2 years, respectively.

Mild effects on body weight and body weight gain were observed in both sexes at the highest dose. These effects were considered transient, since they resolved by study completion. Quantification of body weight decreases at Days 189, 385, and 539 revealed that the magnitudes of the decreases in body weight were less than 10% in males and were above 10% in females (at

12%) only at Day 539. Quantification of decreases in body weight gain over Weeks 1, 1-13, 1-52, and 1-104 revealed that decreased body weight gain never exceeded 10% in males and exceeded 10% (at 11%) in females only at Weeks 1-52. Together, these data indicate that the effects of the test substance on body weight gain and body weight were mildly adverse. No test article effects on food consumption or food efficiency were observed.

No effects of the test article were observed on clinical signs, ophthalmology, clinical chemistry parameters (including coagulation), hematology, urinalysis, organ weights, and gross pathology. There were also no effects of the test article on mortality.

No microscopic effects of the test article were observed in Satellite animals.

The LOAEL for chronic toxicity is 18,000 ppm (892/957 mg/kg bw/day in males/females) based on mild decreases in body weight and body weight gain. The NOAEL is 6,000 ppm (279/309 mg/kg bw/day).

No treatment-related tumors were observed.

This study in the rat is **Acceptable/Guideline** and satisfies the guideline requirements for a Combined Chronic Toxicity/Carcinogenicity Study (OPPTS 870.4300) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality Claim statements were provided.

A flagging statement was also included noting that this study meets or exceed criteria number 1 in EPA 40 CFR part 158.34.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: DPX-MAT28 technical

Description: White powder

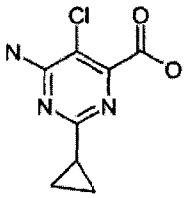
Lot/batch #: DPX-MAT28-23/8571-J

Purity: 90.5% per COA revision 1 on 8/30/2007
88.3% per COA revision 2 on 12/3/2008
*The sponsor-supplied purity is different in the Certificate of Analysis (COA) on revisions 1 and 2 because improved analytical methods were available near the completion of the study. These improved analytical methods are due to improved quantification of inorganic salts and separation of an impurity peak from the active ingredient. This resulted in minimal differences from dietary concentrations based on the initial purity (about 2.2% lower). This falls within the range of analytical variability and the differences between measured and nominal dietary concentrations and so are minimal will not significantly impact study conclusions.

Compound stability: Stability information for the test substance was documented in the Certificate of Analysis which designated the expiration date for the batch of test substance used in this study as August 17, 2010.

CAS # of TGAI: CAS # 858956-08-8

Structure:



2. Vehicle and/or positive control: Not applicable – DPX-MAT28 mixed directly into food

3. Test animals:

Species: Rats - male and female

Strain: Crl:CD(SD)

Age/weight at study initiation: Approximately 6 weeks old;
Weight range for males: 180.4-249.4 g.
Weight range for females: 130.5-186.4 g.

Source: Charles River Laboratories, Japan

Housing: Animals were housed 2 per cage in solid shoe bottom cages during the study.

Diet: Gamma irradiated Lab Diet® #5002, PMI Nutrition International, USA; *ad libitum*

Water: Tap water (municipal water supply filtered and UV irradiated); *ad libitum.*

Environmental conditions:

Temperature:	23±3 °C
Humidity:	40-60%
Air changes:	No information provided
Photoperiod:	12 hrs dark/ 12 hrs light

Acclimation period: 6 days

B. STUDY DESIGN:

1. **In life dates:** November 8, 2007 (In-Life Start)
November 13, 2009 (In-Life Termination).

2. **Animal assignment:** Animals (400/sex) were assigned to either the Main Study (sacrificed at end of 2 yrs) or the Satellite Study (sacrificed at the end of 1 yr) as noted in Table 1.

Table 1 – Group Designation and Dosage						
Test Group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day, M/F)	Main Study		Satellite Study	
			# Male	# Female	# Male	# Female
Group 1 – Control	0	0/0	70	70	10	10
Group 2	600	27.4/29.3	70	70	10	10
Group 3	2000	97.1/99.8	70	70	10	10
Group 4	6000	278.6/308.7	70	70	10	10
Group 5	18000	892.2/957.3	70	70	10	10

3. **Dose selection rationale:** Dietary concentrations of 0, 600, 2000, 6000, or 18000 ppm were selected by the sponsor for use in the 2-year combined chronic toxicity/carcinogeneticity study in rats. The 18000 ppm level was expected to produce non-lethal effects. The 600 ppm level was expected to be a no-observed adverse-effect level (NOAEL). The intermediate concentrations were expected to produce a dose-response for any observed effects. The 18000 ppm level was expected to deliver limit dose dictated by regulatory test guidelines for the combined chronic toxicity and carcinogeneticity study in rats

4. **Diet preparation and analysis:** DPX-MAT28 was mixed with diet using a V-type mixer. The diets were formulated weekly and stored in the refrigerator [approximately 4°C, Archives No.: KIT-401(48), Archives No.: KIT-401(51)] or at room temperature until it was given to the animals.

   All dietary concentrations were collected at the initial diet preparation and analyzed to verify the concentration (average of homogeneity samples) and homogeneity of DPX-MAT28 in the diets. Due to a change in the duration of mixing, homogeneity of the diet preparation was reanalyzed on Day 27 or 34. Dietary samples were taken from the 600 and 18000 ppm concentrations at the initial diet preparation (Day -2) and analyzed to verify the stability of DPX-MAT28 in the diet. Diet samples were collected from all concentrations every 3 months to verify concentration. Duplicate samples were collected from all dietary concentrations. One sample was analyzed and the other was stored frozen for backup. All back up samples were not used for the analyses and disposed after the completion of analysis.

   Results:

   Homogeneity and stability analysis indicated that DPX-MAT28 was homogeneously mixed in the diet at the target concentrations and stable over the test period, as reported in Annex 1 (Analytical Report) of the Study Report:

   Homogeneity analysis: The homogeneity results show that the test substance was mixed homogeneously (CV's = 1.1-3.0, 1.7-4.9. 1.4-4.1 and 1.5-0.6% for 600, 2000, 6000, and 18000 ppm samples, respectively) and at the targeted concentrations (89.1 to 102.3 % of nominal) for all dietary levels.

Concentration analysis: The concentration verification results show that the test substance was at the targeted concentrations (83.4 to 111.4% of nominal) for all dietary levels.

Stability analysis: The stability of the diets at concentrations of DPX-MAT28 is as follows:

Sample Type	600 ppm (% Nominal)	18000 ppm (% Nominal)
0 Day Room Temperature[a]	100.0	100.0
7 Day Room Temperature	103.5	100.9
14 Day Room Temperature	97.3	95.6
14 Day Refrigerated	99.5	99.7
21 Day Refrigerated	98.8	100.8

a - The 0-day room temperature result is the mean result from the top, middle, and bottom homogeneity sample analysis. The homogeneity samples were frozen immediately upon diet preparation and used as baselines for comparison with the stability results.

5. **Statistics:** Statistical analysis for survival and tumor incidence were conducted by Dupont Haskell. Data for survival were prepared by KIT and forwarded to Dupont Haskell as electronic data. The statistical analysis summarized as follow.

Methods of Statistical Analysis			
Parameter	Preliminary Test	If preliminary test is not significant	If preliminary test is significant
Body Weight Body Weight Gain Food Consumption Food Efficiency Clinical Pathology Incidence of Ophthalmology Observations Organ Weight	Bartlett's test for Homogeneity[a]	One-way analysis of variance followed by Dunnett's test	Kruskal-Wallis test followed by Dunn's Rank Sum test
Survival	None	Cochran-Armitage test for trend[b,c] Kaplan-Meier analysis	
Incidence of Microscopic Lesions	None	Cochran-Armitage test for trend[b,d,e] Fisher's exact test Peto analysis[f]	

a - When the Bartlett test indicated no significant deviations from variance homogeneity, the ANOVA multiple comparison test (and Dunnett's test, if necessary) was conducted to determine which pairs of group comparison were significantly different. Significant deviations from variance homogeneity were observed, a non-parametric comparison (Kruskal-Wallis H Test) was conducted. When a significant difference was observed in the Kruskal-Wallis H Test, the Dunn's Rank Sum Test was conducted to determine the specific pairs of group comparison which were significantly different.
b - If the incidence was not significant, but a significant lack of fit occurred, then Fisher's exact test with a Bonferroni-Holm correction was used.
c - The trend test was applied to the data sequentially. If a significant dose-response was detected, data from the top dose group was excluded and the test repeated until no significant trend was detected.
d - If tissues (subject to availability) were not evaluated microscopically in all groups, or if it was unlikely that most tumors were observed grossly, then Fisher's exact test was used.
e - Cochran-Armitage test for trend was applied sequentially to evaluate microscopic observations if all groups were evaluated. Otherwise, Fisher's Exact test was used with a Bonferroni-Holm correction
f - Peto analysis was utilized to assess the significance of a brain neoplasm incidence relative to survival.

C. METHODS:

1. Observations:

1a. Cageside observations: Cageside evaluations to detect moribund or dead rats or acute clinical signs of systemic toxicity, abnormal behavior, and/or appearance among rat were conducted once a day, except on the days when detailed clinical observations were conducted. Additional observations were performed to monitor animals considered to be in poor condition more closely.

1b. Clinical examinations: Each rat was individually handled and examined for abnormal behavior and appearance once in the pre-treatment period (including spare animals), at the first day of dosing prior to test article exposure, and once a week thereafter. Detailed clinical observations in a standardized arena were performed and included (but were not limited to) evaluation of fur, skin , eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling presence of clonic, tonic, stereotypical, or bizarre behavior and presence of masses, etc. Any abnormal clinical sign noted was recorded until the last weekly observation prior to the necropsy for all animals.

1c. Neurological evaluations: No neurological evaluations were performed in addition to the evaluations of autonomic signs and movement described above in 1b. Previous subchronic studies had yielded negative results for FOB parameters.

2. **Body weight:** Body weight was measured twice during the acclimation period (Days -13 and -8) and once during the pre-treatment period (Day -3). During the treatment period, all animals were weighed on Day 0 prior to test article exposure, at the first day of every study week during the first 13 weeks and every other week thereafter and stopped at the last biweekly interval prior to necropsy. Animals were weighed on the days of necropsy.

3. **Food consumption and compound intake:** Individual food consumption of all animals was measured during pre-treatment (Week -1), and weekly throughout the study and calculated weekly during the first 13 weeks, and biweekly thereafter. The amount of food consumed by each rat over each calculating interval was determined by weighing each feeder at the beginning and end of the interval subtracting the final weight and dividing by the number of animals in the cage. From these measurements, mean daily food consumption over the interval was determined. The mean daily food efficiency and mean daily intake of test article were calculated using food consumption and body weight weekly during first 13 weeks and biweekly thereafter. Recording of food consumption was stopped at the last weekly measurement prior to the fasting for urine sampling and necropsy for all animals. Diets containing the test article were given to the animals until they were fasted for necropsy.

4. **Opthalmoscopic examination:** Ophthalmological examinations were conducted by three veterinarians (two veterinarians at each time point) with competency in ophthalmological evaluation designated by KIT Management. Ophthalmological examination on all animals was conducted once during the pre-treatment period (including spare animals). Rats with pre-existing ophthalmological abnormalities were eliminated from the study and replaced by spare animals with normal ophthalmological evaluations. All surviving rats were examined prior to the interim sacrifice and the final sacrifice. Both eyes of each rat were examined by focal illumination and

indirect ophthalmoscope (IO-H, Neitz Instrument Co., Japan) after mydriasis had been produced with Mydrin-P (Santen Pharmaceutical Co., Japan).

5. Hematology and clinical chemistry: Animals were fasted for at least 16 hours prior to blood collection. Blood collected prior to terminal sacrifice was collected from the tail vein. Blood collected at sacrifice was collected from the abdominal *vena cava* of each while the animal was under isoflurane anesthesia.

For the main study animals, blood was collected for possible evaluation of differential WBC counts from all animals sacrificed *in extremis* and from all rats at 12, 18, and 24 months.

For Satellite Study animals, blood smears for WBC counts were performed at Week 26 and 1 year, coagulation was performed at 1 year. The following, CHECKED (X) parameters were examined at Week 26 and 1 year:

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time) [a]		
	(Clotting time)		
X	(Prothrombin time) [a]		

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.
[a] At Week 26 only.

b. Clinical chemistry: Blood samples from satellite animals (approximately 1 mL on week 26 or 3 mL at 1-year necropsy) collected at the same time as for hematology were put into tubes for serum separation, placed at room temperature (approximately 30 min), and then centrifuged (approximately 3000 rpm, 10 min). Serum samples were analyzed for the following parameters:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
	Gamma glutamyl transferase (GGT)*		
X	Sorbitol dehydrogenase*		
	Glutamate dehydrogenase*		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. Urinalysis: : Urine was collected from fasted Satellite Study animals at Week 26 and 1 year. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood/ red blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

7. Sacrifice and pathology: Complete necropsy examinations were performed under the direct supervision of a veterinary pathologist on all animals found dead (tissue integrity permitting), euthanized in extremis, and euthanized at scheduled necropsy (at the 1-year interim sacrifice and 2-year terminal sacrifice). No necropsy was performed on spare animals. Animals were euthanized by isoflurane inhalation and exsanguination. Blood samples were collected from the vena cava while animals were under isoflurane anesthesia. The order of sacrifice for scheduled deaths was stratified among all treatment groups within a sex. The animals were examined carefully for external abnormalities and abnormalities of the abdominal, thoracic and cranial cavities. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)* (includes cerebrum, cerebellum, midbrain, medulla/pons)
X	Salivary glands*	XX	Heart*+	X	Periph.nerve* (sciatic nerve)
X	Esophagus*	x	Bone marrow*	X	Spinal cord (3 levels)* (cervical, mid-thoracic, lumbar sciatic)
X	Stomach*	X	Lymph nodes* (mandibular and mesenteric)	X	Pituitary*
X	Duodenum*	XX	Spleen*+		Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus	X	GLANDULAR
X	Ileum*				Adrenal gland*+
X	Cecum*	X	UROGENITAL		Lacrimal gland
X	Colon*	XXX	Kidneys*+	X	Parathyroids*
X	Rectum*		Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+	X	OTHER
	Gall bladder* (not rat)	XX	Epididymides*+	x	Bone (sternum and/or femur) (femur/knee joint ant sternum)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
	Pancreas*	X	Seminal vesicle*	X	Skin*
X	RESPIRATORY	XX	Ovaries*+ (weighed with oviducts)		All gross lesions and masses*
X	Trachea*	XX	Uterus*+(weighed with cervix)	X	Peyer's patches
X	Lung*++	X	Mammary gland*	X	Eyes (including retina and optic nerve)
X	Nose* (3 levels)	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.
++Organ weight required if inhalation route.
X Collected, but not evaluated.

An initial histopathological evaluation was performed by Greg P. Sykes, VMD, DACVP and peer review was performed by Steven R. Frame, DVM, PhD, DACVP at:
DuPont Haskell Global Centers for Health & Environmental Sciences
PO Box 50
Newark, DE 19714, USA

8. Additional pathology evaluation by a Pathology Working Group (PWG): An additional pathology evaluation was conducted by a PWG organized by Experimental Pathology Laboratories, Inc. (Sterling, VA USA) to review the brain histopathology and interpret the findings. The PWG was composed of 5 independent consulting pathologists with expertise in rodent toxicity and carcinogenicity studies, and with specific expertise in neuropathology. A sixth pathologist with similar background served as the PWG chairperson and author of the final report. All of the brain histopathology slides were independently reviewed by one of the 5 PWG pathologists prior to the PWG meeting to confirm the findings of the DuPont study- and peer-review pathologists. During the PWG meeting, the brain slides with neoplastic findings were coded and reviewed by each panel member. Following this independent review, the panel members reached consensus on the diagnosis for each slide and conducted a weight-of-evidence evaluation to determine if any histopathological findings were spontaneous or associated with test substance administration. The results of the PWG are presented in a separate report in Annex 6 of the Study Report. The original brain diagnoses that were reviewed by the PWG remain

unchanged in this final pathology report, although an alternative diagnosis suggested by the PWG for one of the female brain tumors is discussed in the results section.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No test article-related clinical signs were noted in daily or weekly clinical observations.

   Subdued behavior, thin appearance, irregular respiration, loss of fur, soiled fur, paleness, palpable mass, ulceration, scratch wound, lacrimation, hard skin, scab, and eye discharge were observed in many males and/or females in two or more dose levels during the treatment period. Clinical signs were observed sporadically without any time- or dose-dependency; therefore, they were considered not to be test article-related. Also, these clinical signs were typical for rats of this type of study, age and strain.

2. **Mortality:** There were no test article-related deaths at any concentration, as no test article-related difference in survival was observed among groups within a sex. Mortality during the study period is summarized in Table 2 (obtained from page 36 of the study). None of the differences was statistically significant.

Table 2 – Mortality of Test Subjects*

Group	Dietary Concentration (ppm)	Mortality**			
		Found Dead	Moribund	Total	Survial %
1 Male	0	32	11	43	39
1 Female		8	35	43	39
2 Male	600	16	15	31	56
2 Female		13 (1[a])	31	44(1[a])	37
3 Male	2000	21(1[a])	23	44(1[a])	37
3 Female		12	33	45	36
4 Male	6000	28	19	47	33
4 Female		12	34	46	34
5 Male	18000	21	13	34	51
5 Female		12	36	48	31

* Data obtained from page 36 of the study report
** Mortality was collected on test day 727.
[a] One male in 2000 ppm and one female in 600 ppm were found dead on Days 731 and 733, respectively.

B. BODY WEIGHT AND WEIGHT GAIN:

No significant changes in overall body weight or overall body weight gain were observed in males or females over the 2 year duration of the study (Table 3). There were transient decreases in body weight and body weight gain during the study that had resolved by study termination. These occurred at the highest dose only (18000 ppm) in both males and females. Quantification of body weight decreases at Days 161, 385, and 539 for males and at Days 189, 385, and 539 for females revealed that the magnitudes of the decreases in body weight were less than 10% in males and were above 10% in females (at 12%) only at Day 539. Quantification of decreases in body weight gain over Weeks 1, 1-13, 1-52, and 1-104 revealed that decreased body weight gain never exceeded 10% in males and exceeded 10% (at 11%) in females only at Weeks 1-52. Together, these data indicate that the effects of the test substance on body weight gain and body weight were mildly adverse.

Table 3. Body weights (BW) and body weight gains (BWG)[a] (mean±SD)					
	0	600 ppm	2000 ppm	6000 ppm	18,000 ppm
MALES initial BW	216.3 ±12.82 (N=80)	216.2 ±12.27 (N=80)	216.3 ±11.34 (N=80)	210.4 ±10.40 (N=80)	214.2 ±10.70 (N=80)
Day 161	656.3 ±63.25 (N=79)	664.0 ± 72.90 (N=80)	664.3 ±75.47 (N=79)	647.8 ±81.24 (N=80)	616.8 ±56.79** (↓ 6%) (N=80)
Day 385	813.2±81.11 (N=67)	816.1±86.22 (N=69)	819.7±108.73 (N=61)	820.2±113.91 (N=66)	751.5±81.69** (N=66) (↓ 8%)
Day 539	860.0±115.31 (N=57)	877.0±114.69 (N=59)	870.6±116.75 (N=54)	884.5±155.21 (N=59)	801.9±124.85** (N=63) (↓ 7%)
Final BW	877.3±139.43 (N=27)	879.3±152.83 (N=39)	856.2±170.70 (N=27)	840.9±165.12 (N=25)	788.5±163.19 (N=36)
BWG Wk 1 (% C)	61.6±5.67 (N=80)	60.9±8.56 (N=80)	58.2±6.68** (N=80)	66.4±8.10** (N=80)	59.7 ± 7.61 (N=80)
BWG Wk 1-13 (%C)	349.2 ±48.72 (N=80)	355.1 ±51.80 (N=80)	351.0 ±48.57 (N=80)	344.6 ± 59.0 (N=80)	321.8 ± 40.81** (N=80) (↓8%)
BWG Wk 1-52 (% C)	576.1 ±74.96 (N=78)	579.9 ±79.10 (N=80)	589.2 ±97.36 (N=71)	592.9 ±105.65 (N=76)	523.3±77.22** (N=78) (↓9%)
Overall BWG Wk -1-104	664.4 ±133.59 (N=27)	664.9 ±149.42 (N=39)	641.3 ±168.71 (N=27)	633.0 ±164.28 (N=25)	577.0 ±160.43 (N=36)
FEMALES Initial BW	158.7±9.65 (N=80)	158.6±8.74 (N=80)	157.1±8.94 (N=80)	155.0±8.39* (N=80) (↓2%)	154.2±9.90** (N=80) (↓3%)
Day 189	345.8±40.75 (N=80)	349.5±34.08 (N=80)	349.6±31.11 (N=79)	346.2±32.50 (N=80)	324.2±28.47** (N=79) (↓6%)
Day 385	441.9±80.88 (N=66)	438.3±68.10 (N=69)	441.7±68.40 (N=68)	445.6±53.91 (N=69)	402.7±54.09** (N=66) (↓9%)
Day 539	505.1±111.38 (N=59)	500.2±98.55 (N=52)	503.6±98.90 (N=54)	500.8±73.09 (N=54)	442.5±72.41** (N=56) (↓12%)
Final BW	535.2±153.47 (N=27)	538.7±82.23 (N=27)	533.3±121.84 (N=25)	518.8±86.13 (N=26)	487.3±85.64 (N=23)
BWG Wk 1 (% C)	27.1±6.65 (N=80)	28.5 ±6.64 (N=80)	28.5 ±6.62 (N=80)	30.3 ±5.45** (N=80)	28.9 ±5.47 (N=80)
BWG Wk 1-13 (%C)	140.2±23.73 (N=80)	143.8±19.25 (N=80)	146.5±19.78 (N=80)	145.3±18.77 (N=80)	132.4±17.64* (N=80) (↓6%)
BWG Wk 1-52 (% C)	267.9 ±73.13 (N=76)	268.6 ±56.81 (N=79)	274.3 ±57.15 (N=78)	269.8 ±50.59 (N=79)	239.7 ±48.63* (N=77) (↓11%)
Overall BWG Wk -1-104	379.5 ±150.43 (N=27)	382.3 ±80.87 (N=27)	374.9±120.54 (N=25)	360.2±85.55 (N=26)	332.7±85.30 (N=23)

[a] Data obtained from Tables 6-9, pages 67 -96 of study report.

*Significant differences from control at p<0.01

**Significant differences from control at p<0.01

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:**

No test article-related effects on food consumption and food efficiency were observed in male or female rat at any concentration Mean overall (day 0-721) food consumption in 18000 ppm males and females was 99% and 101% of controls, respectively. Over the first year on study, mean food consumption in this group was 98% and 99% of control in males and females, respectively. None of the differences was statistically significant. Statistically significant differences in mean food consumption were observed over one or more weekly or biweekly intervals in all treated groups. These changes were not considered test article related as there was no consistent pattern of dose response, and they did not result in a significant difference in overall food consumption in these groups.

2. **Compound consumption:** Compound consumption by male and female rats during the study is listed in Table 4.

Table 4. Average Daily Intake of DPX-MAT28 (mg/kg/day)[a]

Treatment Groups		Males	Females	Males	Females	Males	Females
		Day 0-91		Day 0-357		Day 0-539	
Group 1- 0 ppm	Mean	0.0	0.0	0.0	0.0	0.0	0.0
	SD	0.0	0.0	0.0	0.0	0.0	0.0
Group 2- 600 ppm	Mean	37.6	42.9	29.4	33.4	27.4	29.3
	SD	2.39	2.48	2.08	2.87	3.47	2.89
Group 3 – 2000 ppm	Mean	126.1	143.7	98.4	11.5	97.1	99.8
	SD	9.59	7.70	9.60	9.62	18.44	14.62
Group 4 - 6000 ppm	Mean	382.1	440.5	291.4	343.8	278.6	308.7
	SD	30.13	26.47	25.17	32.20	44.61	45.26
Group 5- 18000 ppm	Mean	1147.6	1315.8	924.7	1069.7	892.2	957.3
	SD	76.62	85.05	88.99	108.02	138.41	117.17

[a] Data obtained from Tables 14 and 15pages 134 and 142, respectively of the study report.

3. **Food efficiency:**

Test article-related decreases (relative to control) in food efficiency were observed in male and female rats at 18000 ppm. Mean overall (day 0-721) food efficiency was 87% of control in males and females fed 18000 ppm. Over the first year on study, mean food efficiency in this group was 92% of control in males and 91% of control in females. In both males and females, changes in food efficiency at 2 year interval were not statistically significant, whereas in females (not in males), changes in food efficiency at 1 year interval were statistically significant. Changes in mean food efficiency were consistent with decreased body weight parameters.

D. OPTHALMOSCOPIC EXAMINATION

There were no test article related findings in ophthalmology in both sexes of any dosed group prior to the 1-year interim or the final sacrifice.

E. BLOOD ANALYSIS

1. Hematology and clinical chemistry:

Satellite Animals - There were no treatment related changes in group mean hematology parameters in male or female rats at test days 180/181 or 364/365. There were no statistically significant changes observed in the relative differential counts of white blood cells at test day 364.

Main Study Animals - There were no statistically significant changes observed in the relative differential WBC counts at test days 342 or 728.

F. URINALYSIS

There were no effects of the test substance on urinary parameters.

G. SACRIFICE AND PATHOLOGY

1. Organ Weights

Satellite Animals
Following one year of dietary exposure to DPX-MAT28, there were no test substance-related organ weight effects. All individual and mean organ weight differences were interpreted as spurious and unrelated to test substance dietary exposure.

Main Study Animals
Following 2 years of dietary exposure to DPX-MAT28, there were no test substance-related organ weight effects.

In males, a small decrease in the mean absolute weight of some organs in the 18,000 ppm group was attributed to a test substance-related decrease in the mean terminal body weight (TBW). Decreases in the mean absolute liver (10%), kidneys (11%), heart (7%), and brain (4%) weights were interpreted as secondary to a 10% decrease in the 18,000 ppm TBW, as compared to the control value. Only the heart and brain weight differences were statistically significant. Since the mean relative (% TBW) organ weight values were not decreased and there was no associated gross or microscopic pathology, the decreased mean absolute organ weight values were not considered indicative of any organ specific effect.

Similarly, a statistically significant increase (15%) in the 18,000 ppm mean relative (% TBW) epididymides weight, as compared to the control value, was a result of the decreased mean TBW and not an effect on the epididymides. There was no associated gross and microscopic pathology in this organ.

A statistically significant decrease (3%) in the male 2000 ppm mean absolute brain weight was considered spurious since it was not associated with any test substance-related pathology or body weight effect and was not dose related.

In females, there were no statistically significant differences in organ weight values as compared to the respective control values.

All other individual and mean organ weight differences were interpreted as spurious and unrelated to test substance dietary exposure.

2. Gross pathology

Satellite Animals
There were no test substance-related gross observations recorded at the one-year interim necropsy. All gross observations were consistent with background lesions that are typical of rats of this age and strain.

Main Study Animals
Following 2 years of dietary exposure to DPX-MAT28, there were no test substance-related gross observations.

All gross observations in this study were considered background lesions consistent with incidental and age-related lesions typical of rats of this age and strain.

3. Histopathology

Microscopic Findings:

Satellite Animals - There were no test substance-related microscopic findings observed in rats from the one-year interim sacrifice. All microscopic findings were consistent with normal background lesions in rats of this age and strain. There were no statistically significant ($p<0.05$) increases or decreases in the incidences of any microscopic findings in either sex.

Main Study Animals - Following 2 years of dietary exposure to DPX-MAT28, there were no test substance-related microscopic findings. A slight increase in the incidence of astrocytomas in the 18,000 ppm males was interpreted as spurious.

a. Brain

In males, three 18,000 ppm rats and one 6000 ppm rat were diagnosed with an astrocytoma (Table 6). The incidence of astrocytomas in the 18,000 ppm group (3/69; one brain was unavailable due to cannibalism) was statistically increased ($p<0.05$) by the Cochran Armitage trend test. The incidence was not statistically increased when compared to the control group (0/70) with the Fisher exact Test. A single undifferentiated glioma was observed in an 18,000 ppm male and an oligodendroglioma was observed in a 6000 ppm male. Two granular cell tumors were also observed in the 18,000 ppm males. Granular cell tumors originate in the meninges and not the brain proper.

In females, 5 astrocytomas were observed, including 0/70, 2/70, 1/70, 0/70, and 2/70 in the control, 600, 2000, 6000, and 18,000 ppm concentration groups, respectively (Table 6). One undifferentiated glioma was diagnosed in a 2000 ppm female. (Note: This latter neoplasm was subsequently diagnosed as most likely being a pineal gland carcinoma. Since this

neoplasm is considered incidental, regardless of diagnosis, the data tables have not been modified). In addition, there were 2 neoplasms of meningeal origin: one benign granular cell tumor in a 6000 ppm female and one meningeal sarcoma (i.e., malignant meningeoma) in an 18,000 ppm female.

A Pathology Working Group of 6 independent, board-certified pathologists concluded that the tumors were not treatment-related (Annex 6 of Study Report on Page 4483). The Agency concurs.

s

Table 6 – Incidences of Brain Tumors in Rats Orally Administered DPX-MAT28

Males							
Group	1	2	3	4	5	Historical Control †	
Dosage	0	600	2000	6000	18000	Incidence	Range
# in Group	70	70	70	70	69	2146	
*M Astrocytoma	0	0	0	1	3# (4.3%)	26 (1.2%)	0 - 4.9%
M Glioma, undifferentiated	0	0	0	0	1 (1.4%)	3 (0.14%)	0 - 1.92%
M Oligodendroglioma	0	0	0	1	0	3 (0.14%)	0 – 2.0%
B Granular cell tumor	0	0	0	0	2 (2.9%)	8 (0.37%)	0 – 2.0%
Combined glial tumors	0	0	0	2	4#*		
Females							
Group	1	2	3	4	5	Historical Control ††	
Dosage	0	600	2000	6000	18000	Incidence	Range
# in Group	70	70	70	70	70	2344	
*M Astrocytoma	0	2	1	0	2 (2.86%)	9 (0.38%)	0 – 2.31%
*M Glioma, undifferentiated	0	0	1a	0	0		
*M Meningeal sarcoma	0	0	0	0	1 (1.43%)	1 (0.04%)	0 – 2.0%
*B Granular cell tumor	0	0	0	1	0		
Combined glial tumors	0	2	2	0	2		

Data were obtained from pages 41 and 44 of the study
†Historical control data based on 2146 male brains examined in 30 carcinogenicity studies.
†† Historical control data based on 2344 female brains examined in 31 carcinogenicity studies.
M = malignant; B = benign
Note: There were no statistically significant differences between treated and control groups by Fisher's exact test ($p<0.05$).
Statistically significant ($p<0.05$) by the Cochran-Armitage trend test
* Statistically significant ($p<0.05$) by Peto analysis.
a - Alternatively diagnosed as a pineal carcinoma (non-glial tumor

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

DPX-MAT28 is not a carcinogen in rats. Under the conditions of this study, the no-observed adverse-effect level (NOAEL) for DPX-MAT28 was 6000 ppm for male and female rats, equivalent to 279 and 309 mg/kg/day, respectively. The NOAEL is based on adverse test article related effects on body weight in male and female rats fed 18000 ppm.

The LOAEL can be established at 18000 ppm, or 892 mg/kg/day and 957 mg/kg/day respectively in males and females.

B. REVIEWER COMMENTS:

Five groups of Main male and female Crl:CD(SD) rats (70 /sex/group) were administered DPX-MAT28 in the diet at concentrations of 0, 600, 2000, 6000, or 18000 ppm (0/0, 27/29, 279/309, 892/957 mg/kg bw/day in males/females) for 2 years. Satellite animals (10/sex/dose) were administered DPX-MAT28 at these same dietary concentrations. For both Main and Satellite animals, body weights and food consumption were evaluated prior to dietary exposure, weekly for the first 13 weeks, and then biweekly thereafter. Body weights were also recorded on the day of necropsy. Detailed clinical observations were performed prior to dietary exposure, on the day of first dietary exposure, and weekly thereafter. Ophthalmological assessments were performed prior to the start of dietary exposure and near the end of the exposure period. For Satellite animals, hematology, clinical chemistry, and urinalysis were performed at 6 months and 1 year and coagulation measurements were performed at 1 year. For Main Study animals, blood smears and white blood differential counts were performed at 1 year and 2 years and for animals sacrificed *in extremis*. Satellite and Main Study animals were sacrificed, organ weights were recorded, and gross and histopathology performed at 1 year and 2 years, respectively.

Mild effects on body weight and body weight gain were observed in both sexes at the highest dose. These effects were considered transient, since they resolved by study completion. Quantification of body weight decreases at Days 189, 385, and 539 revealed that the magnitudes of the decreases in body weight were less than 10% in males and were above 10% in females (at 12%) only at Day 539. Quantification of decreases in body weight gain over Weeks 1, 1-13, 1-52, and 1-104 revealed that decreased body weight gain never exceeded 10% in males and exceeded 10% (at 11%) in females only at Weeks 1-52. Together, these data indicate that the effects of the test substance on body weight gain and body weight were mildly adverse. No test article effects on food consumption or food efficiency were observed.

No effects of the test article were observed on clinical signs, ophthalmology, clinical chemistry parameters (including coagulation), hematology, urinalysis, organ weights, and gross pathology. There were also no effects of the test article on mortality.

No microscopic effects of the test article were observed in Satellite animals.

The LOAEL for chronic toxicity is 18,000 ppm (892/957 mg/kg bw/day in males/females) based on mild decreases in body weight and body weight gain. The NOAEL is 6,000 ppm (279/309 mg/kg bw/day).

No treatment-related tumors were observed.

This study in the rat is **Acceptable/Guideline** and satisfies the guideline requirements for a Combined Chronic Toxicity/Carcinogenicity Study (OPPTS 870.4300) in rats.

C. **STUDY DEFICIENCIES** : No deviations/deficiencies were noted in reviewing the study.

DATA EVALUATION RECORD

Aminocyclopyrachlor
PC Code: 28808
TXR#:
MRID#: 48333608

Chronic (1 year) Oral Toxicity Study-Dogs
OPPTS 870.4100

Prepared for

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Quality Control Date 6-1-11
Daniel Ewald

Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-42
EPA Reviewer/WAM: Ryman/Ottley

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Jessica Ryman, Ph.D. **Signature:**
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 10/6/2011
EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10-6-2011
Work Assignment Manager: Lori Brunsman **Signature:**
Science and Information Management Branch, Health Effects Division (7509P) Date:

Template version 02/06

TXR#: 0056049

DATA EVALUATION RECORD

STUDY TYPE: Chronic (1 year) Oral Toxicity Study-Dogs; OPPTS 870.4100; OECD 452.

PC CODE: 288008, 288009, 288010

DP BARCODE: D386645

TEST MATERIAL (PURITY): DPX-MAT28 Technical (Purity: 90.5-88.3%)

SYNONYMS: Aminocyclopyrachlor; 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid, 4-Pyrimidinecarboxylic acid (CAS uninverted); 6-amino-5-chloro-2-cyclopropyl-(CAS inverted); 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid (IUPAC)

CITATION: Han, Su-Cheol Ph.D., (2010), DPX-MAT28 Technical: Chronic Oral Toxicity One-year Feeding Study in Beagle dogs, Korea Institute of Toxicology, Daejeon Korea, Laboratory ID IG07380, October 22, 2010, MRID 48333608. Unpublished.

SPONSOR: E. I. du Pont de Nemours and Company, Wilmington, Delaware 19898 U.S.A.

EXECUTIVE SUMMARY: In a chronic oral toxicity study (MRID 48333608), DPX-MAT28-23 technical (88.3% a.i.; Batch No. 8571-1) was administered to four beagle dogs/sex/dose group in the diet at doses of 0, 1,250, 5,000, 15,000 or 30,000 ppm (equivalent to 0, 37.9, 178.0, 465.1 and 1076.7 mg/kg/day in males and equivalent to 0, 46.9, 174.6, 542.1 and 1072.5 mg/kg/day in females) for 12 months.

There were no treatment related deaths in the study. No adverse treatment-related effects were observed in the following parameters e.g. body weight and body weight gain, food consumption and food efficiency, clinical signs (including neurological parameters), ophthalmology evaluation, hematology (including coagulation), clinical chemistry, urinalysis, gross findings, organ weights and histopathology.

The NOAEL is 30,000 ppm (1076.6/1072.5 mg/kg bw/day in males/females). The LOAEL was not observed.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.4100b; OECD 452) for a chronic oral toxicity study in dogs.

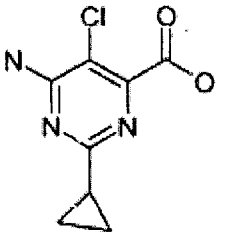
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DPX-MAT28-23

Description: White powder
Batch #: 8571-1
Purity: 88.3 %
Stability of compound: The test compound was stable in the diet for either 7/14 days at room temperature or 14/21 days refrigerated.
CAS #: 858956-08-8
Structure:



2. Vehicle: Diet

3. Test animals:

Species: Dog
Strain: Beagle

Age/weight at dosing: 8-9 months; 6.7-10.7 kg males, 6.0-10.6 kg females
Source: Marshall Beijing, China (No. 6 Da Yang Fang, An Ding Men Wai, Beijing, China
Housing: Individually in pens with partition. Pens were equipped with stainless steel food bowls and water valve.
Diet: A standard certified commercial dog powdered diet (SAFE SAS certified SAFE DIETS, Italy), offered daily for 2 hours in the morning
Water: Tap water, *ad libitum*

Environmental conditions:
Temperature: 22±3°C
Humidity: 55+ 10%
Air changes: Averaged at least 13.43/h
Photoperiod: 12 h light/12 h dark

Acclimation period: 14 days (Prior to the pre-treatment phase)

B. STUDY DESIGN

1. **In life dates:** Start (initiation of dosing): April 23, 2008
End (terminal sacrifice): April 22, 2009

2. **Animal assignment:** The dogs were randomly assigned to the dose groups, based on Path/Tox system using the most recent weight of the animals. Weight variation of the dogs used was targeted not to exceed ±20% of the mean weight for each sex. The randomization of the animals was manipulated so that liter mates were not assigned to the same groups.

TABLE 1. Study design			
Test group	Dietary concentration (ppm)	Males/Females	Compound intake (mg/kg/day, M/F)
1	0	4/4	0/0
2	1250	4/4	37.9/46.9
3	5000	4/4	178/174.6
4	15000	4/4	465.1/542.1
5	30000	4/4	1076.7/1072.5

Data were obtained from page 29 of the study report (MRID 48333608).

3. **Dose-selection rationale:** The dose levels were selected by the sponsor on the basis of the results of previous studies. No further details were provided in the study report.

4. **Dose preparation, administration, and analysis:** The dietary formulations were prepared once every two weeks by mixing an appropriate amount of DPX-MAT28 with the diet in a V-type mixer. The first formulation however was prepared so that it could be administered for 3 weeks and as required for analytical purposes and were stored under 4°C (in the refrigerator). The control formulations were mixed in the same manner as the test substance. Samples were analyzed to confirm concentration, stability and homogeneity. Homogeneity (top, middle, and bottom of mixing bowl) and stability analyses were performed on 7 day and 14 day room temperature from the feeder and 14 day and 21 day refrigerated from the diet mixer. Duplicate samples of all of the different dietary concentrations were obtained from the first diet preparation (Days -15 or -14) and analyzed to confirm the concentration (average of homogeneity samples) and homogeneity of DPX-MAT28 in the diets.

Results

Homogeneity (% RSD): 3.9-6.9%

Stability (% initial): 99.8-116.8 (21 day refrigerated)
100.2-100.3% (7 days at room temperature)

Concentration (mean % nominal) 84.2-107.1% nominal

The analytical data indicated that the mixing procedure was adequate, the test material was stable in the diet during storage and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** The preliminary statistical test used was Barlett's test for homogeneity. This test was used for the following parameters: body weight, body weight gain, food consumption, food efficiency, clinical pathology, incidence of ophthalmology observations and organ weight. When Barlett;s test showed no statistical significant difference then other analysis were performed to determine which pairs of group comparisons were statistically different. (e.g. the ANOVA multiple comparisons test and additionally Dunnetts's test if needed). If however, the Barlett;s test was statistically significant then Kruskal –Wallis H test followed by Dunn's Rank Test was run to determine which pairs of groups were statistically different. Statistical analysis was performed by comparing the different dose groups to the control group using either the Statistical Analysis System (SAS/STAT Version 9.1.3. and 9.2.,

Gary, NC, USA) or the Path/Tox System (ver. 4.2.2, Xybion Medical Systems Corporation, USA).

C. METHODS

1. Observations

a. **Cage-side observations:** Animals were observed for mortality and moribundity at least twice daily (in the morning and the evening). These evaluations included general evaluations of the animals usually 1-2 hours after food removal (i.e. treatment) and usually at the same time (within 2 hours). Any abnormal signs and symptoms observed were recorded.

b. **Clinical examinations:** Detailed clinical observations were performed on all dogs once a week. These examinations included (but were not limited to) evaluation of fur, skin, eyes, mucous membranes, occurrence of secretions and excretions, autonomic nervous system activity (lacrimation, piloerection, and unusual respiratory pattern), changes in gait, posture, response to handling, presence of clonic, tonic, stereotypical, or bizarre behavior and presence of masses.

c. **Veterinary physical/ clinical examinations:** Veterinary physical/ clinical examinations were conducted on all animals prior to randomization and every three months by a veterinarian including the day before the necroscopy.

2. **Body weight:** All dogs were weighed after arrival and in the acclimation period. The dogs were weighed weekly (i.e. Days -28, -21, -14 and -7 in the pre-treatment phase and throughout the study starting at Day 0 continuing each week and finally on the day of necropsy and as required for animal health welfare.

3. **Food consumption and compound intake:** Individual food consumption was measured and recorded daily during the pre-treatment and treatment periods and reported weekly. From the food consumptionand body weight data, weekly group mean food efficiency and group mean daily intake (mg/kg/day) were calculated. For the Day 49 to 56 interval, food consumption, food efficiency and mean daily intake values were calculated for 6 days because Day 50 data were not available due to mistaken deletion. The nature of this mistaken deletion was that a staff member of the KIT computer lab had tried to copy data from the Path/Tox system to an EXCEL spreadsheet in order to test the ability of Path/Tox to calculate food efficiency values. It was during this attempted copy that the Day 50 data were accidentally deleted and could not be recovered.

4. **Ophthalmoscopic examination:** The eyes of all dogs were examined externally once during pre-treatment. In addition, eyes were examined on all dogs during the last week of treatment. The ophthalmic examinations were done using the fundus camera as well as the slit lamp both before and after the dogs were treated with mydriatics. Any abnormal ophthalmic changes observed were recorded.

5. **Clinical pathology evaluation:** Blood samples were collected from all dogs during the pre-treatment phase on Days -35 and Days -28 and urine samples were collected on Days -34 and Days -27 (during weeks -5 and -4). During the study blood samples were collected on

Days 90, 188 and 366 (i.e. during the weeks of 13, 27 and 53) and urine samples were collected on Days 91, 189 and 366. The day prior to the collection of the urine samples all animals were placed in metabolism cages and urine was collected for 16 hours from each dog. Blood samples were collected (for hematology, clinical chemistry, differential WBC count and clinical chemistry) from the cephalic vein from each animal. All blood samples were checked visually for quality.

6. **Hematology and clinical chemistry:** Blood samples were collected from all animals for both hematology and coagulation test. Blood samples were obtained from the animals and placed into tubes that contained EDTA-2K for hematology parameter testing and for the coagulation test the samples were placed into tubes containing 3.2% sodium citrate. The following CHECKED (X) parameters were examined. Blood samples for clinical chemistry were obtained from all dogs and placed into tube without any anti-coagulant for about 90 minutes at room temperature. It was then centrifuged for 10 minutes at approximately 3000 rpm.

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular (cell) HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular cell volume (MCV)
X	Platelet count	X	Absolute Reticulocyte count
X	Blood clotting measurements	X	Red blood cell distribution width (RDW)
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Creatinine
X	Phosphorus	X	Urea nitrogen
X	Potassium	X	Total cholesterol
X	Sodium	X	Globulins
		X	Glucose
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALP)	X	Total protein (TP)
X	Alanine aminotransferase (also SGPT)	X	Triglycerides
X	Aspartate aminotransferase (also SGOT)		
X	Sorbitol dehydrogenase		

7. **Urinalysis:** The CHECKED (X) parameters were examined. The color and clarity of the urine sample were evaluated visually. The urine volume was measured by mass cylinder. The urine pH, ketone, urobilinogen, bilirubiun and occult blood were measured by CliniTek-500, Bayer an automatic tester. The protein and glucose in the urine was measured by TBA200FR and the osmolality was measured by Micro Osmette.

X	Colour	X	Glucose (quantitative test)
X	Volume	X	Ketones
X	Clarity	X	Occult blood
X	Specific gravity / osmolality	X	Bilirubin
X	pH	X	Urobilinogen
X	Protein (quantitative test)	X	Microscopic urine sediment examination

8. Sacrifice and pathology

At study completion all the animals were euthanized by thiopental sodium, on Days 371 or 372 or 373. The CHECKED (X) tissues were collected for microscopic evaluation. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*[d]+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*[e]	X	Spinal cord (cervical, mid-thoracic, lumbar)*
X	Stomach*	X	Lymph nodes* (mandibular and mesenteric)	X	Ear (for animal ID)[g]
X	Duodenum*	XX	Spleen*+	X	Eyes (with optic nerves)*
X	Jejunum*	X	Thymus*+		GLANDULAR
X	Ileum*	X	Peyer's patches*[f]	XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Pituitary
X	Colon*	XX	Kidneys*+	XX	Parathyroid*+
X	Rectum*	X	Urinary bladder*	XX	Thyroid*+
XX	Liver*+[a]	XX	Testes*+		OTHER
X	Pancreas*	XX	Epididymides*+	X	Sternum[e]
		X	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+[c]	X	Femur[e]
X	Trachea*	XX	Uterus*+[b]	X	Skin
X	Lungs*+	X	Mammary gland*		
X	Nasal cavity++	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Recommended for chronic studies based on Guideline 870.4100
\+ Organ weight required in chronic studies.
++ The nasal cavity tissue was preserved with the skull and later processed separately for histopathological findings
a The gall bladder was removed
b The uterus with cervix were weighed together
c The ovaries with oviducts
d Including cerebrum, cerebellum and medulla/pons
e Bone marrow was collected from the femur and the sternum
f The peyer's patches were collected from the sections of the digestive tract
g Not intended for examination by microscope

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity:

No test article-related effects were observed in cageside or clinical examinations (including neurological evaluations).

2. Mortality:

No mortality was observed.

B. BODY WEIGHT AND WEIGHT GAIN:

No test article-related effects on body weight or body weight gain were observed.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

No treatment-related effects on food consumption or food efficiency were observed.

In the male dogs, the overall (day 0-364) average daily intake of the test substance in the 0, 1,250, 5,000, 15,000 and 30,000 ppm groups was 0, 37.9, 178.0, 465.1 and 1076.7 mg/kg body weight/day respectively. In female dogs, average daily intake of the test substance in the 0, 1,250, 5,000, 15,000 and 30,000 ppm groups was 0, 46.9, 174.6, 542.1 and 1072.5 mg/kg body weight/day, respectively.

D. OPHTHALMOSCOPIC EXAMINATION:

No treatment-related effects on opthalmoscopic parameters were observed.

E. BLOOD ANALYSES

No treatment-related effects on hematology or clinical chemistry parameters were observed.

F. URINALYSIS

No treatment-related effects on urinalysis parameters were observed.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** There were no effects of the test substance on absolute and relative organ weights.

2. **Gross pathology:** There were no effects of the test substance on gross pathology. All pathological effects observed were normal for dogs of this breed and age.

3. **Microscopic pathology:** There were no effects of the test substance on microscopic pathology. All microscopic effects observed were normal for dogs of this breed and age.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** In this chronic oral toxicity one year feeding study of DPX-MAT28 in beagle dogs, the no-observed-adverse-effect level (NOAEL) was deemed to be above 30,000 ppm (the highest concentration that was tested in the study). This concentration was found to equivalent to 1077 mg/kg/day in male and 1073 mg/kg/day in female beagle dogs respectively. The NOAEL was based on a lack of treatment related adverse effects on any clinical, anatomic pathology or in-life parameters in both the male and female beagle dogs fed up to 30,000 ppm of DPX-MAT28 for one year.

B. **REVIEWERS' COMMENTS:** In a chronic oral toxicity study (MRID 48333608), DPX-MAT28-23 technical (88.3% a.i.; Batch No. 8571-1) was administered to four beagle dogs/sex/dose group in the diet at doses of 0, 1,250, 5,000, 15,000 or 30,000 ppm (equivalent to 0, 37.9, 178.0, 465.1 and 1076.7 mg/kg/day in males and equivalent to 0, 46.9, 174.6, 542.1 and 1072.5 mg/kg/day in females) for at least 12 months.

There were no treatment related deaths in the study. No adverse treatment-related effects were observed in the following parameters e.g. body weight and body weight gain, food consumption and food efficiency, clinical signs (including neurological parameters), ophthalmology evaluation, hematology (including coagulation), clinical chemistry, urinalysis, gross findings, organ weights and histopathology.

The NOAEL is 30,000 ppm (1076.6/1072.5 mg/kg bw/day in males/females). The LOAEL was not observed.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.4100b; OECD 452) for a chronic oral toxicity study in dogs.

A. **STUDY DEFICIENCIES:** The following deficiency was noted that did not affect the integrity of the study:

On Day 50, food consumption data were mistakenly deleted from the Path/Tox system. The nature of this mistaken deletion was that a staff member of the KIT computer lab had tried to copy data from the Path/Tox system to an EXCEL spreadsheet in order to test the ability of Path/Tox to calculate food efficiency vales. It was during this attempted copy that the Day 50 data were accidentally deleted and could not be recovered.

DATA EVALUATION RECORD
^{14}C-Aminocyclopyrachlor (DPX-KJM44)
PC Code: 288009
TXR#: NA
MRID#: 48333609

Metabolism Study
OPPTS 870-7485

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

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Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-42
EPA Reviewer/WAM: Ryman/Ottley

This review may be altered by EPA subsequent to the contractors' signatures above.

EPA Reviewer: Jessica Ryman, Ph D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10/6/2011
EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10-6-2011
Work Assignment Manager: Lori Brunsman **Signature:**
Science and Information Management Branch, Health Effects Division (7509P) Date:
Template version 02/06

TXR #: 0056049

DATA EVALUATION RECORD

STUDY TYPE: Metabolism in Rats; OPPTS 870.7485 (§85-1); OECD 417

PC CODE: 288009 **DP BARCODE:** D386645

TEST MATERIAL (RADIOCHEMICAL PURITY): ^{14}C-Aminocyclopyrachlor (DPX-KJM44) (99.4%)

SYNONYMS: Aminocyclopyrachlor methyl (DPX-KJM44) Technical; or Methyl 6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylate)

CITATION: Himmelstein, M.W., Ph.D., (2010) ^{14}C-Aminocyclopyrachlor Methyl (DPX-KJM44): Absorption, Distribution, Metabolism, and Elimination in the Sprague-Dawley Rat. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, P.O. Box 50, Newark, Delaware 19714, U.S.A. Laboratory Report No. DuPont-27902, August 3, 2010. MRID 48333609. Unpublished.

Himmelstein, M.W. (2008) ^{14}C-DPX-KJM44: plasma pharmacokinetics and pilot material balance in male and female rats. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-22375, August 18, 2008. MRID 47560024. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.

EXECUTIVE SUMMARY: A previous study (MRID 47560024) characterized the plasma pharmacokinetics of DPX-KJM44 in male and female rats and provided a pilot material mass balance. The purpose of the present study (MRID 48333609) was to confirm the material balance and disposition of DPX-KJM44 and characterize metabolism.

In the first experiment, [pyrimidine-2-^{14}C]-aminocyclopyrachlor methyl (^{14}C -DPX-KJM44) (99.2% radiochemical purity) in 0.5% methylcellulose was administered by a single oral dose via

gavage to 4/sex/dose Sprague-Dawley Crl:CD(SD) rats at 25 mg/kg bw. Animals were placed in metabolic cages where exhaled CO_2 and excreta were collected for 72 hours, at which time animals were sacrificed. Radioactivity was quantified in exhaled CO_2, urine, feces, tissues, and the residual carcass and the metabolite profile determined in urine and feces. Recovery of radioactivity was ≥98% for both sexes. Less than 0.05% of radioactivity was found in any tissue with less than 0.1% observed in the carcass overall. The majority (>85%) of the radioactivity was excreted into the urine. Most (>80%) of this urinary excretion occurred within the first 6 hours. A lesser amount (<5%) was excreted into the feces, with the majority of fecal elimination occurring within the first 24 hours. Negligible (<1%) amounts of radioactivity were expired as CO_2.

In the second experiment, ^{14}C -DPX-KJM44 was administered to 4 rats/sex at 25 mg/kg via gavage to bile duct cannulated rats. Urine, feces, and bile were collected over a 48 hour period. There was not significant elimination of radioactivity in bile. In urine, the majority of the radioactivity was in the form DPX-MAT28 (produced by ester hydrolysis of DPX-KJM44), with less than 1% of non-metabolized DPX-KJM44 parent remaining. In the bile and feces, no unmetabolized DPX-KJM44 was identified with DPX-MAT28 as the only metabolite.

This metabolism study in the rat is classified **acceptable/guideline** and, together with the Tier 1 study MRID 47560024, satisfies the guideline requirement for a metabolism study [OPPTS 870.7485 (§85-1)] in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

Radiolabeled test material ^{14}C -DPX-KJM44

Synonym: [pyrimidine-2-^{14}C]-aminocyclopyrachlor methyl (^{14}C -DPX-KJM44)

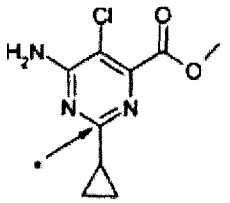
Description: Solid

Radiochemical purity: 99.2% (as of February 14, 2006)

Specific activity: 42.32 µCi/mg, 1.640 x 10^6 Bq/mg[a]

Lot/batch no.: Haskell identification number 22705-145/Stock Item ID 4004667

Structure



* Denotes position of the radiolabel, the position of the radiolabel is considered stable.

Non-radiolabelled test material: DPX-KJM44

Synonym: 4-Pyrimidinecarboxylic acid, 6-amino-5-chloro-2-cyclopropyl-, methyl ester

Description: Solid

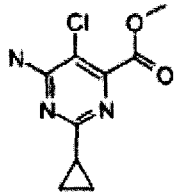
Lot/batch #: Haskell identification number 28240

Purity: 99.4%; (93.27% DPX-MAT28 (aminocyclopyrachlor) acid equivalents on a calculated basis)

Contaminants: None listed as toxicologically significant at this time.

CAS # of TGAI: 858954-83-3

Chemical Structure:



Reference Standard: DPX-MAT28

Synonym: 4-Pyrimidinecarboxylic acid, 6-amino-5-chloro-2-cyclopropyl-

Description: Solid

Lot/batch #: H-28239

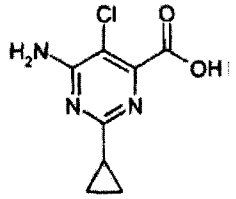
Purity: 99.7%

Contaminants: None listed as toxicologically significant at this time.

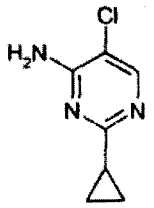
Stability: At least 50 days

CAS # of TGAI: 858956-08-8

Chemical Structure:



Qualitative standard: IN-LXT69 , (used as a qualitative standard for the purpose of identifying potential metabolites)

Synonym:	5-Chloro-2-cyclopropyl-pyrimidin-4-ylamine
Lot/batch #:	22703-449
Purity:	95.0%
CAS # of TGAI:	Not available
Chemical Structure	

2. **Vehicle:** 0.5% methylcellulose

3. **Test animals:**

Species:	Rat (male and female)	
Strain:	Crl:CD(SD) Sprague-Dawley	
Age/ weight at study initiation:	Sexually mature, at least 8 weeks Grouped by ±20% of mean weight by dose group	
Source:	Charles River Laboratories, Inc. (Raleigh, North Carolina)	
Housing:	Individually housed in glass rodent metabolic cages, which allowed separation and collection of urine, feces, and respired $^{14}CO_2$ and volatile metabolites	
Diet:	Certified Rodent LabDiet 5002 chunk chow (PMI Nutrition International, St. Louis, MO); *ad libitum.* Animals were fasted prior to and two hours after single dose experiment.	
Water:	Tap water, *ad libitum*	
Drinking Solution:	Bile-duct cannulated rats, were provided a drinking solution containing dextrose (276 mM), KCl (6.7 mM) and NaCl (154 mM), pH 6.3, to help maintain hydration and bile flow, *ad libitum,* from the day before through the end of 48-hr biliary elimination experiment,	
Environmental conditions	**Temperature:**	18-26°C (64-79°F)
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	12 hours dark/12 hours light
Acclimation period:	At least 6 days At least 3 days for bile-duct cannulated	

4. **Preparation of dosing solutions:** ^{14}C-DPX-KJM44 was diluted with unlabeled DPX-KJM44 to the appropriate specific activity for the selected dose level. The ^{14}C-DPX-KJM44, DPX-KJM44, and dose vehicle (0.5% methylcellulose) were weighed into a vial and mixed to a clear solution or homogeneous suspension.

Prior to administration, samples of each suspension were analyzed for radioactive concentration by liquid scintillation counting (LSC), and for chemical concentration and radiochemical purity by HPLC. After dose administration, each suspension was reanalyzed for radiochemical purity to determine stability.

B. STUDY DESIGN AND METHODS

1. **Study Dates:** In-Life initiated June 9, 2009, In-Life completed July 23, 2009.

2. **Group arrangements and dosing:** Sprague-Dawley rats were assigned to the test groups noted in Table 1. Animals were selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury. The weight variation of selected animals did not exceed ± 20% of the mean weight.

   The objectives of this study included evaluating (1) the disposition and material balance of total ^{14}C residues among tissues and excreta, (2) the elimination of ^{14}C residues in bile, and (3) the profile of potential metabolites in urine, feces, and bile, all after single oral gavage administration.

Table 1. Study Design*

Experiment	Dose Level [a] (mg/kg bw)	Number of Animals		Time of Sacrifice (hours)	Samples
		Male	Female		
Material Balance & Tissue Distribution (Terminal)	0 (Vehicle)	1	1	72	U, F, tissues, carcass
	25	4	4	72	U, F, tissues, carcass, CW & FR, exhaled volatile & CO_2 [b] metabolite profile in U and F [c]
Biliary Elimination	0 (Vehicle)	1	1	48	bile, U, F, CW & FR, GIT, carcass
	25	4	4	48	bile, U, F, CW & FR, GIT, carcass, metabolite profile in bile

*Source: Table, p. 14 of the Study Report
bw = body weight, U = urine, F = Feces, CW = cage wash, FR = feed residue, GIT = gastrointestinal tract tissue and contents
[a] Dosed with 40 µCi/rat of [Pyrimidine-2-^{14}C]DPX-KJM44.
[b] Exhaled volatiles determination was halted at 48 hours because <1 % of dose was measured in exhaled breath for the 0-24 and 24-48 hour collection intervals
[c] Metabolite profile (identification and quantification) confirmation in U and F.

3. **Dosing and sample collection:** The test substance was prepared for administration by oral gavage. This route was chosen because it was most commonly used for toxicity studies of DPX-KJM44. Rats were dosed with approximately 40 µCi/animal (4 mL/kg bw) for the single-dose experiments. Dose solutions were prepared as presented in Table 2.

Table 2. Dosing*					
Dose Level (mg/kg bw)[a]	Dose Volume (mg/kg bw)	Radiochemical Dose (μCi/kg bw)	Specific Activity in Dose (μCi/mg)	Chemical Concentration (mg/mL)	Radiochemical Concentration (μCi/mL)
0 (Vehicle)	0				
25	4	160[b]	6.4	6.25	40

* Source: Table, p. 19 of the Study Report
[a] 0.25 kg body weight/rat
[b] 40 μCi/rat for the single-dose experiments

a. **Pharmacokinetic studies**: Samples were collected at various times depending on the type of study as indicated in Table 1. For sample storage stability representative pooled urine, pooled homogenized feces and pooled bile samples from male rats administered ^{14}C-DPX-KJM44 were evaluated to establish freezer storage stability at <-5°C.

b. **Material Balance and Tissue Distribution:** Four male and four female rats were administered DPX-KJM44 formulated with ^{14}C-DPX-KJM44 at 25 mg/kg bw. One male and one female rat were each administered the dose vehicle (4 mL/kg bw) for collection of control tissue and excreta samples (excluding exhaled breath collection). Rats dosed with the test substance were placed in closed-glass metabolism units for collection of $^{14}CO_2$ and ^{14}C exhaled volatiles. Closed chamber collection continued for up to 48 hours. Urine and feces were collected predose and at 0-6, 6- 12, 12-24, 24-48 and 48-72 hour intervals over dry ice. The exhaled volatiles and CO_2 were collected into traps containing ethylene glycol, 2N NaOH, and water, respectively, at predose and at 0-6, 6-12, 12-24, and 24-48 hour intervals.

At the end of the experiment, rats were sacrificed by CO_2 asphyxiation and exsanguinated.

The following tissues were collected and weighed. The residual carcass was also collected.

Material Balance and Tissue Distribution					
X	blood (plasma & RBC) [a]	X	fat	X	liver
X	kidney	X	muscle	X	heart
X	lung	X	testes	X	ovaries
X	uterus	X	bone and bone marrow [a]	X	brain
X	spleen	X	adrenals	X	pituitary
X	G.I. tract and contents [a]	X	pancreas	X	skin sample
X	thyroid	X	thymus	X	bladder [b]

[a] Analyzed separately for ^{14}C content.
[b] Urine in the bladder at the time of sacrifice was aspirated and placed in the terminal urine sample vial.
Source: p.21 of study report.

Tissues and carcass were stored at less than ≤-10°C until processing and analysis. The remaining carcass was homogenized, stored frozen, and assayed for the determination of material balance.

Over the course of the experiment, residual feed was collected into a single container. It was stored refrigerated (1-10°C) until processing and analysis. After analysis, feed residue homogenized with water was stored frozen (<-10°C). Cages were rinsed with detergent and water (50:50 v/v), water and acetone. Cages were tested to determine efficiency of cage rinse. Cage wash was stored at room temperature.

Exhaled volatile trap solutions, urine, feces, tissues, carcass, cage wash and residual feed were analyzed to determine material balance and tissue distribution. Urine and feces were analyzed for metabolites as described below.

Tissue distribution data were reported as percent of dose, concentration (µg ^{14}C-equivalents/g sample), and tissue-to-plasma concentration ratios. Percentages for tissues that were collected as partial samples (bone, bone marrow, fat, muscle, skin, and whole blood) were adjusted to estimate recovery in the full tissue weight as a percent of the pre-sacrifice body weight.

c. **Biliary Elimination:** Four male and four female rats were administered DPX-KJM44 formulated with ^{14}C-DPX-KJM44 at 25 mg/kg bw by oral gavage. One male and one female rat were each administered the dose vehicle (4 mL/kg bw) for collection of control samples. Following dosing, rats were housed individually in glass metabolism units suitable for the collection of urine and feces.

Urine, feces, and bile were collected on dry ice pre-dose and at approximate intervals of 0-6, 6-12, 12-24, and 24-48 hours post-dose. At the end of the experiment, the rats were killed by $C0_2$ asphyxiation followed by exsanguination. The blood collected by exsanguination was frozen and processed as part of the carcass.

Control samples were used as blanks to check for and minimize the potential for ^{14}C residue carryover when analyzing samples from treated animals. Urine, feces, bile, gastrointestinal tract contents and tissue (analyzed separately), carcass, residual feed, blood, and cage wash were analyzed to determine material balance. Bile was analyzed for metabolites.

i) **Sample preparation and analysis:** Tissues were prepared as detailed in Table 4.

TABLE 3. Sample preparation and analysis

Sample media	Preparation details
Exhaled Volatiles and $^{14}CO_2$	Triplicate aliquots of the ethylene glycol, NaOH and water trap contents were analyzed for ^{14}C by liquid scintillation counting (LSC).
Plasma	Plasma samples, if frozen, were thawed and maintained on wet ice. Generally, aliquots were analyzed by LSC in triplicate (material balance and tissue distribution experiments) using an appropriate volume of the available sample. When ongoing analysis indicated that the ^{14}C concentrations were approaching the limit of quantitation, a single maximum aliquot volume per time point was analyzed.
Urine	Urine samples from each collection interval were thawed, and aliquots analyzed in triplicate for ^{14}C by LSC. Urine samples were submitted for metabolite profiling as described below.
Feces	Feces samples from each collection interval were homogenized in water and aliquots were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total fecal radioactivity for individual rats. Feces samples were submitted for metabolite profiling as described below.
Bile	Bile samples from each collection interval were thawed, and aliquots analyzed in triplicate for ^{14}C by LSC. Bile samples were submitted for metabolite profiling as described below.
Blood	Red blood cells were homogenized and aliquots were combusted. Red blood cells were analyzed using a similar aliquot scheme as noted above for plasma. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total radioactivity present in the tissue.
Cage and container wash	Aliquots of cage rinses were analyzed in triplicate for ^{14}C by LSC.
Tissues	Tissues were homogenized and aliquots were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total radioactivity present in the tissue. For the tissue distribution and multiple-dose experiments, combustion of the smaller tissues was done as single or duplicate aliquots.
Cage Rinses	Aliquots of cage rinses were analyzed in triplicate for ^{14}C by LCS.
Feed Residue	Feed residue was homogenized and aliquots were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC.
Carcass	Carcasses were homogenized, and aliquots of the resulting homogenate were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for $^{14}CO_2$ by LSC.

Source: Data were obtained from pages 22-23 of Study Report.

d. **Metabolite Characterization Studies:** The profiles of ^{14}C-DPX-KJM44 and potential metabolites were evaluated in urine, feces, and bile samples. The objective was to confirm the results from a pilot material balance experiment in which urine and feces were evaluated after 25 mg/kg bw dose administration in a previous study (MRID 47560024). The pilot experiment showed that DPX-KJM44 was absorbed, rapidly metabolized to DPX-MAT28 which was then excreted without further biotransformation. The purpose was to extend the results to include 4 rats/sex for each sample matrix.

i. **Urine:** After LSC assay for determination of radioactivity, aliquots of the urine samples were pooled across the 4 animals per sex per dose group for a given collection interval (0-6, 6-12, 12-24, 24-48, and 48-72 hours) using approximately 25% of the initial sample weight from each collection interval. Pooling through 72 hours was performed to account for greater than 90% of the radioactivity in urine. The concentration of ^{14}C residues in the pooled samples was confirmed by LSC. Samples were stored frozen until further

preparation and analysis. After thawing at room temperature, 0.50 mL of urine was extracted in 0.50 mL methanol by vortexing and centrifuging at 14,000 relative centrifugal force at 20°C. An aliquot (100 µL) was counted by LSC and the remaining sample used for metabolite quantification and identification.

The simultaneous analysis for identification, confirmation and quantitation of metabolites in urine extracts was performed by splitting 0.86 µL/min of the LC effluent directly to the mass spectrometer. Metabolite identities in the radioactive traces were confirmed by direct comparison of LC/MS and LC/RD retention times from the same injection and comparison to reference standards. Structural information was obtained by interpretation of daughter ion scans for each component and comparison to reference standards. Quantitation of the parent and metabolites was done by collecting the remaining effluent in a 96-deep well scintillation plate and analysis on a plate reader.

Each of the prepared urine extracts was also analyzed by LSC to verify adequate extraction and column recoveries. Metabolites were identified based on match of retention time with reference standards.

ii. **Feces:** Feces samples homogenized in water were pooled in the same manner as the urine and counted by oxidation and LSC. The pooled homogenized feces samples were received and stored frozen when not in use. After thawing at room temperature the samples were mixed until homogenous. Approximately 1 gram of sample was placed into a 15-mL centrifuge tube. The sample tubes were then frozen until analysis. After thawing to room temperature a pipet was used to add 4.0 mL of 50:50 methanol:water (v:v) into the 15-mL sample centrifuge tubes. After centrifugation, approximately 1.5 mL supernatant was decanted into a HPLC vial. An aliquot was counted by LSC and the remaining sample was used for metabolite quantification and identification. The feces insoluble pellet was air-dried and analyzed for residual radioactivity via combustion and $^{14}CO_2$ analysis.

Samples were analyzed similar to urine samples.

iii. **Bile:** After LSC assay for determination of radioactivity, aliquots of the bile samples were pooled across the 4 animals per sex per dose group for a given collection interval (0-6, 6-12, 12-24, and 24-48 hours) using approximately 25% of the initial sample weight from each collection interval. Pooling through 48 h was performed to account for greater than 90% of the radioactivity excreted in bile. The concentration of ^{14}C residues in the pooled samples was confirmed by LSC. Samples were stored frozen until further preparation and analysis. After thawing at room temperature, 0.50 mL of bile was transferred into a tared centrifuge tube and the sample weighed. Next, 0.50 mL of methanol was added to the centrifuge tube and the contents vortexed briefly to mix. Approximately 0.80 mL of supernatant was transferred into a HPLC vial. An aliquot (100 uL) was counted by LSC and the remaining sample was used for metabolite quantification and identification.

Samples were analyzed similar to urine samples.

4. **Statistics:** Group data were represented as a mean ± SD. All calculations were carried out with attention to maintaining an adequate number of significant figures so as not to inadvertently misrepresent results. Tables and appendices presented in the report were computer generated and values were rounded appropriately for inclusion in the report. Consequently, the application of manual calculations to report values may in some instances yield a minor variation. These occurrences should not be construed as adversely affecting the integrity or interpretation of the data.

II. RESULTS

A. PHARMACOKINETIC STUDIES

1. **Material Balance:** Material balance results are summarized in Table 4. Exhaled breath samples showed a very low percentage of the dose in the CO_2 trap during the 0-6 hour collection interval; mean values were 0.015% and 0.016% for male and female rats, respectively. All other exhaled breath time intervals and traps through 48 hours were below the limit of detection (LOD = 2x background radioactivity). This finding, except for the detection of the very low radioactivity, was consistent with the pilot experiment data which showed no detectable ^{14}C residues in exhaled breath in 2 rats (1/sex) at 0-24 and 24-48 hours after oral gavage administration of ^{14}C-DPX-KJM44 at 25 mg/kg bw (MRID 47560024). The majority of the dose was excreted in the urine by 24 hours after dose administration, By 72 hours, the mean percent of the dose in urine was 92.4% and 85.1% for male and female rats, respectively. The mean excretion in feces was 4.6% for male rats and 4.4% for female rats. The remainder of recovery was made up of ^{14}C residues in the tissues, carcass, cage wash and feed residue (Table 4). The percent of the dose in the tissues and carcass was minor with a mean value of 0.11% for either sex. More detailed data on tissue distribution are given below. The recoveries in cage wash and feed residue were 1.3% and 0.4% for male rats and 5.0% and 3.35% for female rats, respectively. The overall material balance was excellent with a total mean recovery of 98.7% for male rats and 98.0% for female rats.

Table 4. Overall material balance at 72 hours following a 25 mg/kg bw single oral dose of ^{14}C-DPX-KJM44

		Male	Female
Sample	**Collection Time (h)**	**Mean ± SD (% of dose)**	**Mean ± SD (% of dose)**
Urine	Predose	N.A. ± N.A.	N.A. ± N.A.
	6	85.87 ± 5.42	79.52 ± 7.34
	12	4.62 ± 4.00	2.16 ± 0.92
	24	1.04 ± 0.29	1.75 ± 0.83
	48	0.76 ± 0.43	1.53 ± 1.38
	72	0.08 ± 0.02	0.10 ± 0.07
	Cumulative (72h) percent of dose	92.37 ± 2.07	85.06 ± 5.18
Feces	Predose	N.A. ± N.A.	N.A. ± N.A.
	6	0.08 ± N.A.	0.29 ± N.A.
	12	2.38 ± 1.19	2.47 ± 0.78
	24	1.84 ± 0.76	1.37 ± 0.37
	48	0.29 ± 0.09	0.41 ± 0.17
	72	0.05 ± 0.01	0.11 ± 0.13
	Cumulative (72h) percent of dose	4.59 ± 0.81	4.44 ± 0.55
Exhaled [a]	72	0.015 ± 0.002	0.016 ± 0.002
Cage Wash	72	1.30 ± 0.45	5.04 ± 4.07
Residual feed	72	0.36 ± 0.39	3.35 ± 3.73
Tissue + carcass	72	0.11 ± 0.03	0.11 ± 0.04
Material Balance	72	98.7 ± 1.7	98.0 ± 1.4

[a] Exhaled + CO_2 trap (0-6 hours post-dose), no activity
Source: Data extracted from Tables 2 and 3, pp. 37-38 of study report.

[a] Exhaled + CO_2 trap (0-6 hours post-dose), no activity
Source: Data extracted from Tables 2 and 3, pp. 37-38 of study report.

The distribution of ^{14}C residues was evaluated as the percentage of the administered dose, concentration of ^{14}C equivalents per gram of tissue and tissue:plasma concentration ratios (Table 5). The results indicated extensive systemic distribution of ^{14}C residues; however, the remaining residues were very low by 72 hours after dose administration. The skin, fat, gastrointestinal contents and muscle were the 4 tissues that each contained mean percentages between 0.01% and 0.02% of the dose for either sex. The other tissues had substantially lower or non-detectable percentages. Mean tissue concentrations were all below 0.1 ng ^{14}C-equivalents per gram. The resulting tissue:plasma concentration ratios ranged from 1:1 to 12:1. The 12:1 ratio should be interpreted as a conservative estimate because it was based on a measured concentration in the bladder of only one male rat while the other 3 male rats in the group had concentrations <LOD. The residual activity in the bladder was most likely from urine not completely aspirated from the bladder at necropsy. None of the female rats had detectable radioactivity in the bladder. Overall, the tissue:plasma concentration ratios indicated a very low potential for accumulation of ^{14}C-DPX-KJM44 residues. These results at 72 hours after dose administration for the current experiment were consistent the pilot experiment in which one male and one female rat had

^{14}C residues in systemic tissues that were all <LOD at 168 hours after single dose administration (MRID 47560024).

Table 5. Percent of dose, concentration recovered in tissues and tissue:plasma concentration ratio at terminal sacrifice (72 hours) at terminal sacrifice (72 hours) following a 25 mg/kg bw single oral dose of ^{14}C-DPX-KJM44

	Male			Female		
Sample	Mean ± SD % of dose	Mean ± SD (µg equivalent/g)	Tissue:Plasma Concentration Ratio	Mean ± SD % of dose	Mean ± SD (µg equivalent/g)	Tissue:Plasma Concentration Ratio
carcass[a]	0.0885 ± 0.0290	0.0260 ± 0.0084	3.9	0.0805 ± 0.0235	0.0246 ± 0.0069	3.3
skin[b]	0.0210 ± 0.0060	0.0240 ± 0.0060	3.6	0.0171 ± 0.0007	0.0207 ± 0.0008	2.8
whole blood[b]	0.0026 ± 0.0003	0.0077 ± 0.0007	1.2	0.0025 ± 0.0003	0.0076 ± 0.0010	1.0
Plasma	---	0.0066 ± N.A.	1.0	---	0.0074 ± N.A.	1.0
rbc	---	0.0081 ± 0.0004	1.2	---	0.0080 ± 0.0013	1.1
bone marrow[b]	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.
brain	0.0003 ± 0.0000	0.0100 ± 0.0009	1.5	0.0005 ± 0.0001	0.0125 ± 0.0013	1.7
fat[b]	0.0149 ± 0.0036	0.0466 ± 0.0101	7.1	0.0153 ± 0.0040	0.0505 ± 0.0142	6.9
heart	0.0002 ± 0.0001	0.0133 ± 0.0038	2.0	0.0003 ± N.A.	0.0203 ± N.A.	2.8
lungs	0.0004 ± 0.0000	0.0167 ± 0.0024	2.5	0.0005 ± 0.0001	0.0198 ± 0.0009	2.7
spleen	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.
liver	0.0038 ± 0.0007	0.0193 ± 0.0017	2.9	0.0035 ± 0.0003	0.0195 ± 0.0022	2.6
kidney	0.0008 ± 0.0001	0.0187 ± 0.0013	2.8	0.0012 ± 0.0004	0.0284 ± 0.0102	3.9
G.I. tract	0.0019 ± 0.0005	0.0194 ± 0.0037	2.9	0.0021 ± 0.0004	0.0209 ± 0.0038	2.8
G.I. contents	0.0131 ± 0.0054	0.0517 ± 0.0536	7.8	0.0166 ± 0.0150	0.0612 ± 0.0668	8.3
pituitary	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.
thyroid	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.
thymus	0.0002 ± N.A.	0.0210 ± N.A.	3.2	N.A. ± N.A.	N.A. ± N.A.	N.A.
ovaries	---	---	---	N.A. ± N.A.	N.A. ± N.A.	N.A.
testes	0.0004 ± 0.0001	0.0089 ± 0.0015	1.3	---	---	---
pancreas	0.0002 ± N.A.	0.0154 ± N.A.	2.3	0.0002 ± N.A.	0.0140 ± N.A.	1.9
adrenals	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.
uterus	---	---	---	N.A. ± N.A.	N.A. ± N.A.	N.A.
muscle[b]	0.0150 ± 0.0017	0.0081 ± 0.0005	1.2	0.0147 ± 0.0025	0.0084 ± 0.0014	1.1
bladder	0.0003 ± N.A.	0.0761 ± N.A.	11.6	N.A. ± N.A.	N.A. ± N.A.	N.A.
bone[b]	0.0023 ± 0.0005	0.0102 ± 0.0017	1.5	0.0027 ± N.A.	0.0123 ± N.A.	1.7
Total	0.0765 ± 0.0046			0.0715 ± 0.0206		

[a] Percent of dose recovered in carcass is not included in total.

[b] Percentages for tissues collected as partial samples were adjusted to estimate recovery in the full tissue weight as percent of terminal body weight: skin (19%), whole blood (7.4%), bone marrow (2.3%), fat (7.0%), muscle (40.4%), and bone (5%). (Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., and Beliles, R.P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health 13(4), 407-484).

Source: Data extracted from Tables 4 and 5, pp. 39-40 of study report.

2. **Bilary Elimination:** Rats with bile duct cannulae were administered a^{14}C-DPX-KJM44 25 mg/kg bw dose to measure the extent of absorption based on the percent of dose recovered in bile, urine, carcass, and the gastrointestinal tract tissue (excluding contents) up to 48 hours after administration. The mean total recovery of absorbed and unabsorbed (feces, cage wash, and GI tract contents) radioactivity accounted for 91.7% and 93.2% of administered radioactivity for male and female rats, respectively (Table 6). The majority of the mean absorbed dose was recovered in the urine (87.2% and 87.1%) with only minor amounts recovered in the carcass + GI tract tissue (0.13% and 0.26%) and bile (0.60% and 0.46%). By summation, the mean percent of absorbed dose was 87.9% and 87.8% for male and female rats, respectively.

Comparison of the estimated absorption from the bile duct cannulated rats was consistent with the cumulative excretion in urine of non-bile duct cannulated animals from the material balance experiment with 92.3% and 85.0% at 48 hours for male and female rats, respectively (Table 6).

Table 6. Percent of Dose absorbed based on biliary elimination and material balance following a single 25 mg/kg bw single oral dose of ^{14}C-DPX-KJM44

		Male	Female
Sample	**Collection Time (h)**	**Mean ± SD % of dose**	**Mean ± SD % of dose**
Urine	Predose	N.A. ± N.A.	N.A. ± N.A.
	6	82.388 ± 3.403	85.090 ± 1.731
	12	3.551 ± 2.923	0.992 ± 0.276
	24	0.823 ± 0.445	0.514 ± 0.198
	48	0.424 ± 0.107	0.478 ± 0.350
	Subtotal	87.186 ± 0.241	87.074 ± 1.676
Feces	Predose	N.A. ± N. A.	N.A. ± N.A.
	6	0.006 ± N. A.	0.364 ± N. A.
	12	0.815 ± 0.942	2.041 ± 1.088
	24	2.092 ± 0.763	1.368 ± 0.492
	48	0.255 ± 0.069	0.355 ± 0.072
	Subtotal	2.962 ± 0.450	3.946 ± 0.946
Bile	Predose	N.A. ± N.A.	N.A. ± N.A.
	6	0.563 ± 0.202	0.424 ± 0.086
	12	0.0158 ± 0.0025	0.0165 ± 0.0014
	24	0.0106 ± 0.0004	0.0105 ± 0.0006
	48	0.0075 ± 0.0006	0.0074 ± 0.0004
	Subtotal	0.597 ± 0.204	0.456 ± 0.084
Cage Wash	48	0.697 ± 0.385	0.931 ± 0.536
Residual feed	48	0.135 ± 0.128	0.482 ± 0.195
Carcass[a]	48	0.131 ± 0.025	0.256 ± 0.097
G.I. tract	48	0.0023 ± 0.0003	0.0031 ± 0.0007
Carcass + G.I. tract	48	0.134 ± 0.025	0.259 ± 0.098
G.I. contents	48	0.0271 ± 0.0060	0.0507 ± 0.0180
	Total	91.7 ± 0.8	93.2 ± 0.9
	Absorbed[b]	87.9 ± 0.2	87.8 ± 1.6

[a] Carcass includes whole blood collected at time of sacrifice
[b] Absorbed is sum of dose recovered in urine, bile, carcass + G.I. tract tissue
Source: Table 6, p. 41 of study report.

B. METABOLITE CHARACTERIZATION STUDIES:

The profiles of ^{14}C-DPX-KJM44 and potential metabolites were evaluated in urine, feces, and bile samples. The objective was to confirm the results from a pilot material balance experiment in which urine and feces were evaluated after 25 mg/kg bw dose administration (MRID 47560024). The pilot experiment showed that DPX-KJM44 was absorbed, rapidly metabolized to DPX-MAT28 which was then excreted without further biotransformation. The purpose was to extend the results to include 4 rats/sex for each sample matrix.

The results of this study show that DPX-KJM44, when administered by single oral gavage, is excreted in urine, feces and bile as the free acid DPX-MAT28 (Tables 7 and 8). In the pilot experiment, DPX-MAT28 was also shown to be the sole metabolite of DPX-KJM44 in plasma of rats administered a single 500 mg/kg bw oral dose of ^{14}C-DPX-KJM44 (MRID 47560024). These results indicate very rapid ester cleavage upon first-pass metabolism in the liver and/or systemic circulation. No additional biotransformation of DPX-MAT28 was evident under conditions of the previous pilot experiment or the current study. Therefore, the sum of metabolites quantified in either matrix during the respective collection intervals were nearly equivalent to the sum of ^{14}C residues excreted by the respective excretion route. The metabolic pathway shows one step for biotransformation of DPX-KJM44 to DPX-MAT28 (Figure 1).

Table 7. Summary of components quantified in urine, feces and bile of rats administered ^{14}C-DPX-KJM44

Identified component	Parent or metabolite as percent of dose					
	Male			Female		
	Urine	Feces	Total (U+F)	Urine	Feces	Total (U+F)
DPX-KJM44	0.10	N.A.	0.098	0.08	N.A.	0.080
DPX-MAT28[a]	91.01	4.49	95.50	83.76	4.23	88.00
Identified	91.10	4.49	95.60	83.84	4.23	88.08
Not identified	1.17[b]	0.00	1.17	1.11[b]	0.00	1.11
Unextracted	N.A.	0.013	N.A.	N.A.	0.012	N.A.
Total	92.27	4.51	96.78	84.95	4.25	89.20

[a] Radio-chromatographic retention time was 3.00 minutes using HPLC.
[b] The aggregate number of unidentified components (and the maximum percent of dose for any given component) in urine was 12 (≤0.455%) for male rats and 13 (≤0.364%) for female rats.
Source: Data extracted from Table 9, p. 43 of study report.

Table 8. Summary of components quantified in bile of rats administered ^{14}C-DPX-KJM44 as percent of dose

Identified component	Collection time (hr)	Male	Female
		Bile	Bile
DPX-KJM44	0-6	0.003[a]	0.000
DPX-MAT28[a]	0-6	0.448	0.384
Identified		0.451	0.384
Not identified		0.101[b]	0.040[b]
Unextracted		---	---
Total		0.552	0.424

[a] Although retention time match in radio-chromatogram for male rat bile corresponded to DPX-KJM44, the amount was too low for confirmation by mass spectral analysis,
[b] The aggregate number of unidentified components (and the maximum percent of dose for any given component) in bile was 7 (<0.037%) for male rats and 3 (SO.021%) for female rats.
Source: Data extracted from Table 10, p. 43 of study report.

60

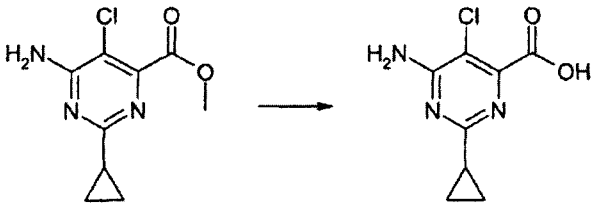


Figure 1. Proposed metabolic pathway of DPX-KJM44 in the rat (reproduced from Figure 22, page 70 of the study report)

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** Material balance was excellent with mean values of 98.7% and 98.0% for male and female rats, respectively. Exhaled breath samples showed a very low percentage of the dose in the CO_2 trap during the 0-6 hour collection interval; the mean values were 0.015% and 0.016% for male and female rats, respectively. By 72 hours after dose administration, the body burden (tissue + carcass) was very low and accounted for 0.11% of the dose in either sex. The majority of the dose was excreted in the urine within 24 hours after administration and by the end of experiment accounted for 92.4% and 85.1% of the dose for male and female rats, respectively. The mean excretion in feces was 4.6% for male rats and 4.4% for female rats. The dose recoveries in cage wash and feed residue were 1.3% and 0.4% for male rats and 5.0% and 3.35% for female rats, respectively. No sex difference was evident in the overall excretion pattern.

The evaluation of tissue distribution demonstrated systemic uptake of ^{14}C-DPX-KJM44 based on quantifiable residues in tissues at 72 hours after oral dose administration. The remaining residues were very low. The skin, fat, gastrointestinal contents and muscle were the 4 tissues that each contained mean percentages between 0.01% and 0.02% of the dose for either sex. The other tissues had substantially lower or non-detectable levels. Mean tissue concentrations were all below 0.1 μg ^{14}C-equivalents per gram. The resulting tissue:plasma concentration ratios ranged from 1:1 to 12:1. The highest ratio was observed in the bladder which only had detectable ^{14}C residues in one male rat most likely because of carryover from radioactivity in residual urine not completely removed at necropsy; the residues in bladders of the other male and female rats were <LOD. Overall, the tissue:plasma concentrations ratios, concentrations and percent of dose data for the evaluation of tissue distribution indicated a very low potential for accumulation of ^{14}C-DPX-KJM44 residues. These results at 72 hours after dose administration for the current experiment were consistent with the pilot experiment in which one male and one female rat had ^{14}C residues in systemic tissues that were all <LOD at 168 hours after single dose administration (MRID 47560024).

The percent of absorbed dose was measured in bile-duct cannulated rats for up to 48 hours after oral dose administration. The majority of the mean absorbed dose was recovered in the urine (87.1% and 87.2%) with only minor amounts recovered in the carcass + GI tract tissue (0.13% and 0.26%) and bile (0.60% and 0.46%). By summation, the mean percent of absorbed dose was 87.9% and 87.8% for male and female rats, respectively. Comparison of the estimated absorption from the bile-duct cannulated rats was consistent with the cumulative excretion in urine of non-bile duct cannulated animals from the material balance experiment: 92.3% and 85.0% at 48 hours for male and female rats, respectively.

The analysis of urine, feces and bile for potential metabolites clearly showed DPX-MAT28, the ester cleavage product of DPX-KJM44 as the only metabolite. A very low percentage of parent DPX-KJM44 was quantifiable in male and female rat urine and male rat bile shortly (0-6 hours) after dose administration. No additional metabolism of DPX-KJM44 was evident under conditions of the current study.

B. REVIEWER COMMENTS: A previous study (MRID 47560024) characterized the plasma pharmacokinetics of DPX-KJM44 in male and female rats and provided a pilot material mass balance. The purpose of the present study (MRID 48333609) was to confirm the material balance and disposition of DPX-KJM44 and characterize metabolism.

In the first experiment, [pyrimidine-2-^{14}C]-aminocyclopyrachlor methyl (^{14}C -DPX-KJM44) (99.2% radiochemical purity) in 0.5% methylcellulose was administered by a single oral dose via gavage to 4/sex/dose Sprague-Dawley Crl:CD(SD) rats at 25 mg/kg bw. Animals were placed in metabolic cages where exhaled CO_2 and excreta were collected for 72 hours, at which time animals were sacrificed. Radioactivity was quantified in exhaled CO_2, urine, feces, tissues, and the residual carcass and the metabolite profile determined in urine and feces. Recovery of radioactivity was ≥98% for both sexes. Less than 0.05% of radioactivity was found in any tissue with less than 0.1% observed in the carcass overall. Rather, the majority (>85%) was excreted into the urine. Most (>80%) of this urinary excretion occurred within the first 6 hours. A lesser amount (<5%) was excreted into the feces, with the majority of fecal elimination occurring within the first 24 hours. Negligible (<1%) amounts of radioactivity were expired as CO_2.

In the second experiment, ^{14}C -DPX-KJM44 was administered to 4 rats/sex at 25 mg/kg via gavage to bile duct cannulated rats with urine, feces, and bile collected over a 48 hour period. There was not significant elimination of radioactivity in bile.

In urine, the majority of the radioactivity was in the form DPX-MAT28 (produced by ester hydrolysis of DPX-KJM44), with less than 1% of non-metabolized DPX-KJM44 parent remaining. In the bile and feces, no unmetabolized DPX-KJM44 was identified with DPX-MAT28 as the only metabolite.

C. STUDY DEFICIENCIES: None.

DATA EVALUATION RECORD

^{14}C-Aminocyclopyrachlor (DPX-MAT28)
PC Code: 288008
TXR#: NA
MRID#: 48333610

Metabolism Study
OPPTS 870-7485

Prepared for

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Quality Control — Date 5-26-11
Daniel Ewald

Contract Number: EP-W-10013
Work Assignment No.: WA-0-01

Task No.: 0-1-42
EPA Reviewer/WAM: Ryman/Ottley

This review may be altered by EPA subsequent to the contractors' signatures above.

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EPA Reviewer: Abdallah Khasawinah, Ph.D. **Signature:**
Registration Action Branch 4, Health Effects Division (7509P) **Date:** 10-6-2011
Work Assignment Manager: Lori Brunsman **Signature:**
Science and Information Management Branch, Health Effects Division (7509P) Date:
Template version 02/06

TXR #: 0056049

DATA EVALUATION RECORD

STUDY TYPE: Metabolism in Rats; OPPTS 870.7485 (§85-1); OECD 417

PC CODE: 288008 **DP BARCODE:** D386645

TEST MATERIAL (RADIOCHEMICAL PURITY): ^{14}C-Aminocyclopyrachlor (DPX-MAT28) (99.5%)

SYNONYMS: [pyrimidine-2-^{14}C]-aminocyclopyrachlor (^{14}C -DPX-MAT28)

CITATION: Himmelstein, M.W., Ph.D., (2010) ^{14}C-Aminocyclopyrachlor (DPX-MAT28): Absorption, Distribution, Metabolism, and Elimination in the Sprague-Dawley Rat. E.I. du Pont de Nemours and Company, DuPont Haskell Global Centers for Health & Environmental Sciences, P.O. Box 50, Newark, Delaware 19714, U.S.A.. Laboratory Report No. DuPont-27389, August 3, 2010. MRID 48333610. Unpublished.

Himmelstein, M.W. (2008) ^{14}C-DPX-MAT28: plasma pharmacokinetics and pilot material balance in male and female rats. E.I. du Pont de Nemours and Company, Dupont Haskell Global Centers for Health & Environmental Sciences, Newark, DE. Laboratory Project ID: DuPont-22033, August 18, 2008. MRID 47560023. Unpublished.

SPONSOR: E.I. du Pont de Nemours and Company Wilmington, Delaware 19898 U.S.A.

EXECUTIVE SUMMARY: A previous study (MRID 47560023) characterized the plasma pharmacokinetics of DPX-MAT28 in male and female rats after administration of a single low or high dose and provided a pilot material mass balance.

In metabolism studies (MRID 48333610), [pyrimidine-2-^{14}C]-aminocyclopyrachlor (^{14}C -DPX-MAT28) (99.5% radiochemical purity) was administered in 0.5% methylcellulose by oral gavage

to male and female Sprague-Dawley Crl:CD(SD) rats (4/sex/dose). Control animals (1/sex/dose) were administered 0.5% methylcellulose. Different study designs were employed to investigate metabolism and disposition.

In the first study, ^{14}C -DPX-MAT28 was administered at a single dose of 25 or 500 mg/kg bw. Total recovery and recovery in urine, feces, and as exhaled CO_2 were monitored for 72 hours. Total recovery was excellent at ≥97.4%. Negligible amounts were exhaled as CO_2. Recoveries in urine and feces were similar for both sexes and doses (with feces being slightly higher) and was near-complete by 72 hours. Recoveries in urine peaked at 6 hours, whereas recoveries in feces peaked at 12-24 hours. At 72 hours, percent recoveries in urine were 47.74 ± 3.75 and 40.04 ± 5.14 for males at 25 and 500 mg/kg bw. For females, these values were 56.51 ± 14.55 and 44.08 ± 4.78, respectively. Percent recoveries in feces at 72 hours were 47.97 ± 3.32 and 54.76 ± 5.14 for males at 25 and 500 mg/kg bw. For females, these values were 39.48 ± 13.42 and 51.08 ± 3.33and 56.51 ± 14.55, respectively.

In the second study, biliary elimination was examined by administration of a single dose of 25 or 500 mg/kg bw in bile duct-cannulated rats for 48 hours. Total recovery and recovery in urine, feces, and bile were monitored for 48 hours. Total recovery was excellent at ≥95.1%. Maximum recoveries and times of peak recoveries in urine and feces were similar to uncannulated rats. However, a larger fraction of radioactivity was increased in the feces than the urine. At 48 hours, percent recoveries in feces were 62.06 ± 3.41 and 57.86 ± 7.77 for males at 25 and 500 mg/kg bw and 68.47 ± 6.59 and 60.68 ± 4.87, respectively, for females. In urine at 48 hours, percent recoveries were 34.52 ± 3.57 and 30.06 ± 7.89 at 25 and 500 mg/kg bw for males and 22.18 ± 2.13 and 32.24 ± 5.60, respectively for females. Maximum excretion in the bile for both sexes occurred by 6 hours. However, total excretion in the bile within 48 hours was low at less than 0.3% for both sexes at both doses. Together, this data indicate a minor role for enterohepatic circulation in the metabolism of ^{14}C -DPX-MAT28.

The tissue distribution of radiolabel was observed at T_{MAX} (1 hour), T_{MAX} + 5 hours, and 72 hours following a single oral (gavage) dose of 25 or 500 mg/kg bw in males/females. The majority of the dose was found in the GI contents (46.7-67.0%) and GI tract (9.54-13.08%) in both sexes at both doses by T_{MAX} + 5 hours without an apparent dose-related increase. Radioactivity was largely absent in the GI contents/GI tract by 72 hours (<<1%). The highest levels or radiolabel for both sexes and doses were found in the carcass (up to about 5%), followed by the muscle (up to 2%), skin (up to 1.4%), and blood (up to 1%). Again, there was no apparent dose-related increase in tissue levels. However, levels in all of these tissues declined to very low levels (<0.5%) by 72 hours. The apparent lack of dose-related increase in the tissue content of radiolabel suggested saturation of uptake.

Tissue distribution after repeated dosing the 25 mg/kg bw/day for 14 days was also examined. On Day 13 at 6 hours following dose administration, Levels were again highest in the GI tract (0.16-0.22%) and GI contents in males/females (7.11-1.14%), but were reduced in magnitude compared to 6 hours following single-dose administration of 25 mg/kg in the GI tract (1.36-1.42%) and GI contents (41.7% and 46.77%) in both sexes. Tissue distribution was similar with carcass > skin > blood, with all levels less than 0.5% within 6 hours of dosing and less than 0.05% by 72 hours.

The analysis of urine, feces and bile for potential metabolites clearly showed that ^{14}C-DPX-MAT28 was the sole ^{14}C component in excreta. No metabolism was evident.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485 (§85-1)] in rats.

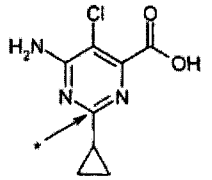
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

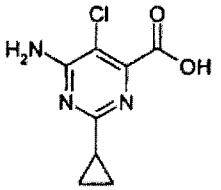
1. Test compound

Radiolabeled test material 1: ^{14}C -DPX-MAT28

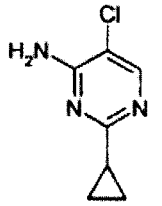
Synonym:	[pyrimidine-2-^{14}C]-aminocyclopyrachlor (^{14}C -DPX-MAT28)
Description:	White solid
Radiochemical purity:	99.5% (as of February 28, 2007) by HPLC/UV, KFT and GC
Specific activity:	42.42 µCi/mg, 1.570 x 10^6 Bq/mga
Lot/batch no.:	2420CEO002-1
Stability:	At least 50 days
Structure	

* Denotes position of the radiolabel.

Non-radiolabelled test material: DPX-MAT28

Synonym:	4-Pyrimidinecarboxylic acid, 6-amino-5-chloro-2-cyclopropyl-
Description:	Solid
Lot/batch #:	D100095-073
Purity:	98.0%
Contaminants:	None listed as toxicologically significant
Stability:	At least 50 days
CAS # of TGAI:	858956-08-8
Chemical Structure:	

Qualitative standard: IN-LXT69 (22703-449), (used as a qualitative standard for the purpose of identifying potential metabolites)

Synonym:	5-Chloro-2-cyclopropyl-pyrimidin-4-ylamine
Lot/batch #:	Not available
Purity:	95.0%
CAS # of TGAI:	Not available
Chemical Structure	

2. Vehicle: 0.5% methylcellulose

3. Test animals:

Species:	Rat (male and female)
Strain:	Sprague-Dawley Crl:CD(SD)

Age/ weight at study initiation:	8 weeks old Grouped by ±20 of mean weight by dose group	
Source:	Charles River Laboratories, Inc. (Raleigh, North Carolina)	
Housing:	Individually housed in glass rodent metabolic cages, which allowed separation and collection of urine, feces, and respired $^{14}CO_2$ and volatile metabolites	
Diet:	Certified Rodent LabDiet 5002 Chunk (PMI Nutrition International, St. Louis, MO); *ad libitum* Animals were fasted prior to and two hours after single dose experiment. Animals were not fasted for the multiple-dose experiment	
Water:	Tap water, *ad libitum*	
Drinking Solution:	Bile-duct cannulated rats were provided a drinking solution *ad libitum* containing dextrose (276 mM), KCl (6.7 mM) and NaCl (154 mM) to help maintain hydration and bile flow, from the day before through the end of 48-hr biliary elimination experiment.	
Environmental conditions	**Temperature:**	18-26°C (64-79°F)
	Humidity:	30-70%
	Air changes:	Not reported
	Photoperiod:	12 hours dark/12 hours light
Acclimation period:	At least 6 days At least 3 days for bile-duct cannulated	

4. **Preparation of dosing solutions:** The ^{14}C-DPX-MAT28 was diluted with unlabelled DPX-MAT28 to the appropriate specific activity for the selected dose level. The ^{14}C-DPX-MAT28, DPX-MAT28, and dose vehicle (0.5% methylcellulose) were weighed into a vial and mixed to a clear solution or homogeneous suspension. The dose levels were based on the results of a toxicity study with DPX-MAT28 and match those used in the pharmacokinetic study. The pH of the low-dose (25 mg/kg bw) preparations were checked and adjusted to approximately pH 7 using sodium hydroxide (NaOH). This was done to facilitate preparation of homogenous dosing solutions free of precipitated test substance. The high-dose preparation was expected to be a suspension. The pH of the high-dose (500 mg/kg bw) preparations were checked and adjusted using a similar amount of NaOH as used for the low dose.

B. STUDY DESIGN AND METHODS

1. **In life dates:** Start: May 12, 2009; End: August 28, 2009.

2. **Group arrangements:** Sprague-Dawley rats were assigned to the test groups noted in Table 1. Animals were selected for use on study based on adequate body weight gain and freedom from any clinical signs of disease or injury. The weight variation of selected animals did not exceed ± 20% of the mean weight.

   The objectives of this study included evaluating (1) the disposition and material balance of total ^{14}C residues among tissues and excreta, (2) the percentage and concentration of ^{14}C residues in tissues at selected times after dosing (Tmax, Tmax+5 hours, and terminal sacrifice), (3) the elimination of ^{14}C residues in bile, and (4) the profile of potential metabolites in urine, feces, and bile, all after single oral gavage administration. Additionally, material balance, tissue distribution and metabolite profiles in urine and feces were studied after repeated oral gavage administration (14 days of low dose administration with 72 hour post-dose follow up after the last dose).

Table 1. Study Design*

Experiment	Dose Level [a] (mg/kg bw)	Number of Animals		Time of Sacrifice (hours)	Samples
		Male	Female		
Material Balance & Tissue Distribution (Terminal)	0 (Vehicle)	1	1	72	U, F, tissues, carcass
	25	4	4	72	U, F, tissues, carcass, CW & FR, exhaled volatile & CO_2 [b] metabolite profile in U and F
	500	4	4	72	U, F, tissues, carcass, CW & FR, metabolite profile in U and F [c]
Biliary Elimination	0 (Vehicle)	1	1	48	bile, U, F, CW & FR, GIT, carcass
	25	4	4	48	bile, U, F, CW & FR, GIT, carcass, metabolite profile in bile
	500	4	4	48	bile, U, F, CW & FR, GIT, carcass, metabolite profile in bile
Tissue Distribution (Tmax)	25	4	4	1 [d]	tissue, carcass
	500	4	4	1 [d]	tissue, carcass
Tissue Distribution (*Tmax + 5 h*)	25	4	4	1 [e]	tissue, carcass
	500	4	4	1 [e]	tissue, carcass
Multiple dose	25 (14 days) [f]	24	6	Various times [g]	Tissue and excreta from various sampling times groups (3 rats/time point) described in-life section below [g, h]

bw = body weight, U = urine, F = Feces, CW = cage wash, FR = feed residue, TBD = to be determined,
GIT = gastrointestinal tract tissue and contents, Tmax = time at maximum plasma concentration (Cmax)

a Dosed with 40 µCi/rat of [Pyrimidine-2-^{14}C]DPX-MAT28.
b Exhaled volatiles determination was halted at 48 hours because <1 % of dose was measured in exhaled breath for the 0-24 and 24-48 hour collection intervals
c Metabolite profile (identification and quantification) confirmation in U and F.(1)
d Tmax based on experimental pharmacokinetic data.(1)
e Tmax + 5 hours was based on experimental pharmacokinetic data.(1)
f Dosed with approximately 6.25 µCi/250 g rat/day of [Pyrimidine-2-^{14}C]DPX-MAT28.
g Tissues were collected at 54, 150, 246, 318, 336, 342, 360, and 384 hours of the experiment.
h Excreta, cage wash, and feed residue were collected from male and female rats (3/sex) throughout the multiple dose experiment for rate and extent of excretion and material balance. Metabolite profiling in urine and feces was performed at 3 collection intervals (0-24, 120-144, and 264-288 hours).

*Source: Table, p. 16 of the Study Report.

3. **Dosing and sample collection:** The test substance was prepared for administration by oral gavage. This route was chosen because it was most commonly used for toxicity studies of DPX-MAT28. Rats were dosed with approximately 40 µCi/animal for the single-dose experiments, and approximately 6.25 µCi/animal per daily dose for the multiple-dose experiment. Dose solutions (Table 2) were prepared as follows:

Table 2. Dosing*

Dose Level (mg/kg bw)[a]	Dose Volume (mg/kg bw)	Radiochemical Dose (µCi/kg bw)	Specific Activity in Dose (µCi/mg)	Chemical Concentration (mg/mL)	Radiochemical Concentration (µCi/mL)
0 (Vehicle)	0				
25	4	160[b]	6.4	6.25	40
500	4	160[b]	0.32	125	40
25	2	25[c]	1.0	12.5	12.5

[a] 0.25 kg body weight/rat
[b] 40 µCi/rat for the single-dose experiments
[c] 6.25 µCi/rat per daily dose for the multiple-dose experiment
* Source: Table, p. 22 of the Study Report.

a. Pharmacokinetic studies

i). Material Balance and Tissue Distribution: Male and female rats were administered DPX-MAT28 formulated with ^{14}C-DPX-MAT28 at 25 and 500 mg/kg bw (4/sex/dose). One male and one female rat were each administered only the dose vehicle (4 mL/kg bw) for collection of control tissue and excreta samples (excluding exhaled breath collection). Rats dosed with the test substance at the low (25 mg/kg bw) dose were placed in closed-glass metabolism units for collection of $^{14}CO_2$ and ^{14}C exhaled volatiles. Closed chamber collection continued for up to 48 hours. Closed chamber collection was not conducted for the high-dose (500 mg/kg bw) group based on results of the low-dose group, which showed no detectable ^{14}C residues in exhaled breath. Urine and feces were collected predose and at 0-6, 6- 12, 12-24, 24-48 and 48-72 hour intervals over dry ice. The exhaled volatiles and CO_2 were collected into traps containing ethylene glycol, 2N NaOH, and water, respectively, at predose and at 0-6, 6-12, 12-24, and 24-48 hour intervals.

At the end of the experiment, rats were sacrificed by CO_2 asphyxiation and exsanguinated through cardiac puncture.

The following tissues were collected and weighed. The residual carcass was also collected.

Material Balance and Tissue Distribution					
X	blood (plasma & RBC) [a]	X	fat	X	liver
X	kidney	X	muscle	X	heart
X	lung	X	testes	X	ovaries
X	uterus	X	bone and bone marrow [a]	X	brain
X	spleen	X	adrenals	X	pituitary
X	G.I. tract and contents [a]	X	pancreas	X	skin sample
X	thyroid	X	thymus	X	bladder [b]

[a] Analyzed separately for ^{14}C content.
[b] Urine in the bladder at the time of sacrifice was aspirated and placed in the terminal urine sample vial.

Tissues and carcass were stored at less than -10°C until processing and analysis.

ii). Biliary Elimination: Bile duct cannulated male and female rats were administered DPX-MAT28 formulated with ^{14}C-DPX-MAT28 at 25 and 500 mg/kg bw (4/sex/dose). One male and one female rat were each administered the dose vehicle (4 mL/kg bw) for collection of control tissue and excreta samples (excluding exhaled breath collection). Following dosing, rats were housed individually in glass metabolism units suitable for the collection of urine and feces.

Urine, feces, and bile were collected on dry ice pre-dose and at approximate intervals of 0-6, 6-12, 12-24, and 24-48 hours post-dose. At the end of the experiment, the rats were killed by $C0_2$ asphyxiation followed by exsanguination. The blood collected by exsanguination was frozen and processed as part of the carcass.

Control samples were used as blanks to check for and minimize the potential for ^{14}C residue carryover when analyzing samples from treated animals. Urine, feces, bile, gastrointestinal tract contents and tissue (analyzed separately), carcass, residual feed, blood, and cage wash were analyzed to determine material balance. Bile was analyzed for potential metabolites.

iii). Tissue Distribution (Tmax and Tmax+5 hours): Eight male and 8 female rats (4 rats/sex/time point) were administered the test substance formulated with ^{14}C-DPX-MAT28 at a dose rate of 25 mg/kg bw. An additional 8 male and 8 female rats were administered the test substance formulated with ^{14}C-DPX-MAT28 at a dose rate of 500 mg/kg bw. Following dosing, rats were housed individually in solid bottom cages lined with absorbent padding or in glass metabolism units suitable for containing ^{14}C residues excreted in urine and feces. Excreta were not saved for analysis.

Tissues and carcass were analyzed to determine ^{14}C tissue distribution. Data were reported as percent of dose, concentration (μg ^{14}C-equivalents/g sample), and tissue-to-plasma ratios. Percentages for tissues that were collected as partial samples (bone, bone marrow, fat, muscle, skin, and whole blood) were adjusted to estimate recovery in the full tissue weight as a percent of the pre-sacrifice body weight.

The following tissues were collected at sacrifice:

Tissue Distribution (Tmax and Tmax+ 5 hours)					
X	blood (plasma & RBC) [a]	X	fat	X	liver
X	kidney	X	muscle	X	heart
X	lung	X	testes	X	ovaries
X	uterus	X	bone and bone marrow [a]	X	brain
X	spleen	X	adrenals	X	pituitary
X	G.I. tract and contents [a]	X	pancreas	X	skin sample
X	thyroid	X	thymus	X	bladder [b]

[a] Analyzed separately for ^{14}C content.
[b] Urine in the bladder at the time of sacrifice was aspirated and discarded as radioactive waste.
Source: p. of the Study Report.

iv. Multiple-Dose Experiment: A multiple dose experiment was conducted to evaluate the tissue distribution, material balance and metabolite profile in urine and feces after repeated oral gavage administration. Thirty rats (24 males and 6 females) were used according the design presented in Table 3.

Table 3. Design of Multiple-Dose Experiment

Day of Experiment	Dose time (hr)	Sac time (hr)	Number of Rats and Sex									
			3M	3M	3M	3M	3F	3M	3M	3M	3M[a]	3F[a]
0	0		D	D	D	D	D	D	D	D	D	D
1	24		D	D	D	D	D	D	D	D	D,U,F[b]	D,U,F[b]
2	48	54	D,S	D	D	D	D	D	D	D	D,U,F	D,U,F
3	72			D	D	D	D	D	D	D	D,U,F	D,U,F
4	96			D	D	D	D	D	D	D	D,U,F	D,U,F
5	120			D	D	D	D	D	D	D	D,U,F	D,U,F
6	144	150		D,S	D	D	D	D	D	D	D,U,F[b]	D,U,F[b]
7	168				D	D	D	D	D	D	D,U,F	D,U,F
8	192				D	D	D	D	D	D	D,U,F	D,U,F
9	216				D	D	D	D	D	D	D,U,F	D,U,F
10	240	246			D,S	D	D	D	D	D	D,U,F	D,U,F
11	264					D	D	D	D	D	D,U,F	D,U,F
12	288					D	D	D	D	D	D,U,F[b]	D,U,F[b]
13	312	318 (6)[c]				D,Sa	D,Sa	D	D	D	D,U,F	D,U,F
14		336 (24)[c]						S			U,F	U,F
		342 (30)[c]							S			
15		360 (48)[c]								S	U,F	U,F
											Sa,U,F	Sa,U,F
16		384 (72)[c]									CW,FR	CW,FR

[a] These animals were housed in glass metabolism units, while all others were maintained in suspended stainless steel rack cages.

[b] Urine and feces samples from 0-24, 120-144, and 264-288 hours were used for metabolite profiling,

[c] Hours after last dose

bw - body weight; CW - Cage wash; D - Dose administration 25 mg/kg bw/day, approximately 6.25 µCi/rat/day, at approximately 2 mL/kg bw; U - Urine; F - Feces (with samples from 0-24; FR - Feed residue; S - Sacrifices with collection of selected tissues (whole blood, plasma, red blood cells, liver, kidney, fat, and muscle); Sa - Sacrifices with full tissue and carcass collection and analysis (same as those collected for the single dose tissue distribution experiments)

Source: Table, p. 26 of the Study Report.

The collection of excreta samples, tissues, cage wash, feed residue, and all other necessary procedures followed those used for the material balance and tissue distribution experiments.

v.). Sample preparation and analysis: Tissues were prepared as detailed in Table 4.

TABLE 4. Sample preparation and analysis [a]

Sample media	Preparation details
Exhaled Volatiles and $^{14}CO_2$	Triplicate aliquots of the ethylene glycol, NaOH and water trap contents were analyzed for ^{14}C by liquid scintillation counting (LSC).
Plasma	Plasma samples, if frozen, were thawed and maintained on wet ice. Generally, aliquots were analyzed by LSC in triplicate (material balance and tissue distribution experiments) using an appropriate volume of the available sample. When ongoing analysis indicated that the ^{14}C concentrations were approaching the limit of quantitation, a single maximum aliquot volume per time point was analyzed.
Urine	Urine samples from each collection interval were thawed, and aliquots analyzed in triplicate for ^{14}C by LSC. Urine samples were submitted for metabolite profiling as described below.

TABLE 4. Sample preparation and analysis [a]

Sample media	Preparation details
Feces	Feces samples from each collection interval were homogenized in water and aliquots were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total fecal radioactivity for individual rats. Feces samples were submitted for metabolite profiling as described below.
Bile	Bile samples from each collection interval were thawed, and aliquots analyzed in triplicate for ^{14}C by LSC. Bile samples were submitted for metabolite profiling as described below.
Blood	Red blood cells were homogenized and aliquots were combusted. Red blood cells were analyzed using a similar aliquot scheme as noted above for plasma.
Cage and container wash	Aliquots of cage rinses were analyzed in triplicate for ^{14}C by LSC.
Tissues	Tissues cells were homogenized and aliquots were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for ^{14}C by LSC to determine total radioactivity present in the tissue. For the tissue distribution and multiple-dose experiments, combustion of the smaller tissues was done as single or duplicate aliquots.
Residual carcass	Carcasses were homogenized, and aliquots of the resulting homogenate were combusted. The CO_2 liberated from combustion was trapped and analyzed in triplicate for $^{14}CO_2$ by LSC.

Source: Data were obtained from pages 26-27 of Study Report.

b. **Metabolite characterization studies:** Radio-chromatograms for male and female rat urine samples after single dose and during multiple dose administration of ^{14}C-DPX-MAT28 showed a single peak. Representative LC/MS reconstructed ion chromatograms of the male and female rat urine (0-6 hour pool) from after single dose administration showed a single peak for DPX-MAT28.

DPX-MAT28 was the sole identified component in urine and represented 47.35% and 56.06% of the single low (25 mg/kg bw) dose and 39.80% and 43.61% of the high (500 mg/kg bw) dose for male and female rats, respectively. After multiple dose administration, DPX-MAT28 in urine of male rats was 38.29%, 39.20% and 38.77% at the 0-24, 120-144, and 264-288 hour collection intervals, respectively. In the urine of female rats, the values were 41.48%, 37.69%, and 31.99% at the same collection intervals. These data indicated an overall range of 32-56% of administered DPX-MAT28 excreted in urine after single low, high or multiple low dose administration. The magnitude of excretion appeared to decline with increasing dose after single oral administration. The percent of excretion during multiple dose administration was most similar to that observed after single high dose administration.

Radio-chromatograms for male and female rat feces samples after single dose and during multiple dose administration of ^{14}C-DPX-MAT28 showed a single peak. Like the analysis of the urine sample above, the identity of DPX-MAT28 was confirmed in feces by comparison of the full scan and daughter ion mass spectra observed in the 6-12 hour pooled male feces sample with the same peak observed in a control feces sample fortified with DPX-MAT28.

Like urine, DPX-MAT28 was the sole identified component in feces and represented 47.43% and 39.24% of the single low (25 mg/kg bw) dose and 51.71% and 55.94% of the high (500 mg/kg bw) dose for male and female rats, respectively. After multiple dose administration, DPX-MAT28 in feces of male rats was 45.06%, 53.75% and 54.53% at the 0-24, 120-144, and 264-288 hour collection intervals, respectively. In the feces of female rats, the values were 43.86%, 54.03%, and 55.20% at the same collection intervals. These data indicated an overall range of 39-56% of administered DPX-MAT28 excreted in feces after single low, high or multiple low dose administration. The magnitude of excretion showed a clear increase with increasing dose after single oral administration. The percent of excretion during multiple dose administration was most similar to that observed after single high dose administration.

Radio-chromatograms for male and female rat bile samples after single dose administration of ^{14}C-DPX-MAT28 showed a single peak.

Like urine and feces but at a much lower percentage of the total dose, DPX-MAT28 was the sole identified component in bile and represented 0.213% and 0.093% of the single low (25 mg/kg bw) dose and 0.153% and 0.100% of the high (500 mg/kg bw) dose for male and female rats, respectively. These data indicated an overall range of 0.093-0.213% of administered -39- DPX-MAT28 excreted in bile after single low and high dose administration. The magnitude of excretion was essentially the same at both dose levels.

The reference standard, IN-LXT69, was not detected in excreta. No match was found for the reference standard IN-LXT69 in any urine, feces or bile samples from the current study.

4. **Statistical analysis:** Data were reported as means ± SD with attention to adequacy of significant figures.

II. RESULTS

A. PHARMACOKINETIC STUDIES

1. **Material Balance:** Radioactive residues were below the limit of detection (LOD = 2x background radioactivity) in exhaled breath samples collected after administration of the low (25 mg/kg bw) dose. This finding confirms and extends the pilot experiment data showing that ^{14}C-DPX-MAT28 residues do not undergo excretion by this route.

   The majority of the dose was excreted between the urine and feces in a roughly proportional manner. Excretion was substantially complete in the first 24 hours after dose administration at both the low (25 mg/kg bw) and high (500 mg/kg bw) dose levels. By 72 hours after dose administration, the mean percent of the dose in urine ranged from 40.0% to 56.5% with a slight reduction evident at the high (500 mg/kg bw) dose relative to the low (25 mg/kg bw) dose. The mean excretion in feces ranged from 39.5% to 54.8% of the dose with a slightly higher percentage excreted at the high (500 mg/kg bw) than low (25 mg/kg bw) dose. This finding confirms and extends the pilot experiment data showing that ^{14}C-DPX-MAT28 residues do not undergo excretion by this route.

   The majority of the dose was excreted between the urine and feces in a roughly proportional manner. Excretion was substantially complete in the first 24 hours after dose administration at both the low (25 mg/kg bw) and high (500 mg/kg bw) dose levels. By 72 hours after dose administration, the mean percent of the dose in urine ranged from 40.0% to 56.5% with a slight reduction evident at the high (500 mg/kg bw) dose relative to the low (25 mg/kg bw) dose. The mean excretion in feces ranged from 39.5% to 54.8% of the dose with a slightly higher percentage excreted at the high (500 mg/kg bw) than low (25 mg/kg bw) dose. Mean percent of dose recovered for mass balance following a single dose is presented in Table 5.

Table 5. Percent of dose recovered following a 25 or 500 mg/kg bw single oral dose of ^{14}C-DPX-MAT28

		25 mg/kg bw		500 mg/kg bw	
		Male	Female	Male	Female
Sample	Collection Time (h)	Mean ± SD (% of dose)	Mean ± SD (% of dose)	Mean ± SD (% of dose)	Mean ± SD (% of dose)
Urine	Predose	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.
	6	35.75 ± 7.09	45.12 ± 13.00	22.14 ± 10.83	31.93 ± 2.89
	12	5.02 ± 0.64	5.17 ± 1.85	11.59 ± 11.35	4.28 ± 1.69
	24	5.30 ± 4.38	4.79 ± 2.78	4.36 ± 0.87	5.39 ± 3.12
	48	1.56 ± 0.76	1.13 ± 0.56	1.78 ± 0.50	2.11 ± 0.89
	72	0.11 ± 0.04	0.30 ± 0.22	0.19 ± 0.05	0.37 ± 0.20
	Cumulative (72h) percent of dose	47.74 ± 3.75	56.51 ± 14.55	40.04 ± 5.14	44.08 ± 4.78
Feces	Predose	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.
	6	0.18 ± 0.25	0.65 ± 0.37	0.76 ± N.A.	N.A. ± N.A.
	12	23.98 ± 7.46	22.68 ± 14.42	18.19 ± 12.8	10.74 ± N.A.
Feces	24	19.86 ± 5.84	14.95 ± 3.01	29.16 ± 9.79	35.06 ± 13.77
Feces	48	3.67 ± 2.90	1.17 ± 0.32	6.49 ± 1.88	9.79 ± 11.54
Feces	72	0.27 ± 0.13	0.19 ± 0.06	0.53 ± 0.26	0.85 ± 0.83
Feces	Cumulative (72h) percent of dose	47.97 ± 3.32	39.48 ± 13.42	54.76 ± 5.14	51.08 ± 3.33
Exhaled [a]	72	<LOD ± N.A.	<LOD ± N.A.	N.A. ± N.A.	N.A. ± N.A.
Cage Wash	72	2.43 ± 1.78	2.87 ± 1.63	1.84 ± 0.35	2.08 ± 1.12
Residual feed	72	0.328 ± 0.228	0.316 ± 0.08	0.648 ± 0.411	0.243 ± 0.149
Tissue + carcass	72	0.064 ± 0.047	0.222 ± 0.120	0.085 ± 0.046	0.184 ± 0.071
Material Balance	72	98.5 ± 1.2	99.4 ± 0.7	97.4 ± 0.3	97.7 ± 1.2

[a] Exhaled volatiles were only measured in the single low (25 mg/kg bw) experiment.
Source: Extracted from Tables 2 through 4, pp. 46-48 of study report.

2. **Bilary Elimination:** Rats with bile duct cannulae were administered ^{14}C-DPX-MAT28 to measure the extent of absorption based on the percent of dose recovered in bile, urine, carcass, and the gastrointestinal tract tissue (excluding contents) up to 48 hours after administration. The mean total recovery of absorbed and unabsorbed (feces, cage wash, and GI tract contents) radioactivity accounted for 95.1 to 98.0% of administered radioactivity at the 25 or 500 mg/kg bw dose levels. The majority of the mean absorbed dose was recovered in the urine (22.2 to 34.5%) with only minor amounts recovered in the carcass + GI tract tissue (0.09 to 0.66%) and bile (0.13 to 0.25%). By summation, the mean percent of absorbed dose was 34.9% and 22.4% at the low (25 mg/kg bw) dose and 30.9% and 32.6% at the high (500 mg/kg bw) dose for male and female rats, respectively.

The estimated absorption from the bile duct cannulated rats was low compared with the cumulative excretion in urine of non-bile duct cannulated animals from the material balance experiment (39.9% to 56.2% of the dose administered at 48 hours after single low or high dose administration). In the bile duct cannulated rats, the majority of the dose was excreted un-absorbed in the feces (57.9 to 68.5%), suggesting that conditions within the

biliary elimination experiment reduced gastrointestinal tract absorption. A possible explanation is that the combination of the intake of the drinking solution (measured pH 6.3) containing dextrose (276 mM), KCl (6.7 mM) and NaCl (154 mM) to help maintain hydration and bile flow and the DPX-MAT28 (pKa 4.65)[4] dose preparation being pH balanced to approximately pH 7.0 acted to reduce absorption from the gastrointestinal tract. In this case, the urinary excretion + tissue + carcass from the single and multiple dose material balance experiments appears to provide a better estimate of the absorbed dose, accounting for 37 to 57% by 72 hours after dose administration. Mean percent of dose recovered for biliary elimination and mass balance following a single dose is presented in Table 6.

Table 6. Percent of dose observed based on biliary elimination and material balance following a single 25 or 500 mg/kg bw single oral dose of ^{14}C-DPX-MAT28

		25 mg/kg bw		500 mg/kg bw	
		Male	Female	Male	Female
Sample	Collection Time (h)	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose
Urine	Predose	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.
	6	20.69 ± 6.28	14.95 ± 2.82	18.37 ± 5.01	15.87 ± 2.53
	12	6.80 ± 1.31	3.58 ± 2.96	5.28 ± 2.08	7.08 ± 1.56
	24	4.27 ± 1.83	2.23 ± 1.57	4.12 ± 2.13	6.50 ± 2.18
	48	2.75 ± 1.60	1.42 ± 0.21	2.29 ± 1.69	2.80 ± 2.23
	Subtotal	34.52 ± 3.57	22.18 ± 2.13	30.06 ± 7.89	32.24 ± 5.60
Feces	Predose	N.A. ± N. A.	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.
	6	0.00 ± N.A.	0.28 ± 0.39	0.03 ± 0.04	5.34 ± 7.09
	12	31.88 ±7.73	44.08 ± 6.25	6.49 ± 4.59	13.52 ± 9.12
	24	22.89 ± 7.43	28.97 ± 20.57	31.88 ± 7.75	23.81 ±8.90
	48	7.29 ± 3.65	6.16 ± 5.33	21.10 ± 6.57	31.39 ± 15.33
	Subtotal	62.06 ± 3.41	68.47 ± 6.59	57.86 ± 7.77	60.68 ± 4.87
Bile	Predose	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A. ± N.A.
	6	0.170 ± 0.044	0.093 ± 0.027	0.121 ± 0.055	0.072 ± 0.007
	12	0.043 ± 0.009	0.030 ± 0.008	0.032 ± 0.008	0.028 ± 0.010
	24	0.023 ± 0.006	0.013 ± 0.006	0.023 ± 0.014	0.020 ± 0.014
	48	0.012 ± 0.004	0.005 ± 0.003	0.006 ± 0.003	0.009 ± 0.006
	Subtotal	0.25 ± 0.03	0.14 ± 0.02	0.18 ± 0.07	0.13 ± 0.03
Cage Wash	48	0.42 ± 0.18	3.64 ± 5.74	5.77 ± 6.00	1.21 ± 0.68
Residual feed	48	0.16 ± 0.05	0.49 ± 0.29	2.39 ± 1.25	0.59 ± 0.36
Carcass[c]	48	0.08 ± 0.04	0.10 ± 0.07	0.64 ± 0.42	0.18 ± 0.08
G.I. tract	48	0.018 ± 0.012	0.005 ± 0.003	0.017 ± 0.013	0.018 ± 0.020
Carcass+ G.I. tract	48	0.09 ± 0.04	0.10 ± 0.7	0.66 ± 0.42	0.20 ± 0.08
G.I. contents	48	0.48 ± 0.23	0.11 ± 0.07	0.36 ± 0.35	0.63 ± 0.53
	Total	98.0 ± 0.7	95.1 ± 0.3	97.3 ± 0.3	95.7 ± 1.5
	Absorbed[b]	34.9 ± 3.6	22.4 ± 2.1	30.9 ± 8.0	32.6 ± 5.6

[a] Carcass includes whole blood collected at time of sacrifice
[b] Absorbed is sum of dose recovered in urine, bile, carcass + G.I. tract tissue
Source: Table 5, p. 49 of study report.

3. **Tissue distribution:** The distribution of ^{14}C residues was evaluated as the percentage of the administered dose,[1] concentration of ^{14}C equivalents per gram of tissue, and tissue:plasma concentration ratios at Tmax (1 hr), Tmax+5 (6 h), and terminal sacrifice (72 h) after single dose administration. The majority of the dose was associated with the gastrointestinal tract contents and subsequently showed systemic uptake and distribution to all the tissues. Elimination was rapid between the 6 and 72 hour sample times such that ^{14}C residues were below the limit of quantitation (LOQ = 3x background radioactivity) by 72 hours in the majority of the tissues. Low percentages were evident among the skin, kidney, GI tract tissue

[1] Percentages for tissue collected as partial tissues were adjusted to estimate recovery in the full tissue. This adjustment precluded the percentage in the carcass from being used in the calculation of the total tissue burden. Therefore, the adjusted total tissue burden will be qualitative similar to but will not match the measured percent of dose in the carcass + tissues reported for the material balance experiment.

and GI contents. The tissue concentration data and tissue:plasma concentration ratios indicated no potential for bioaccumulation because ^{14}C residues were no longer quantifiable at the terminal collection time (72 hours) after dose administration. For all single dose groups, the mean total tissue burdens, which ranged from 67% to 81% of the dose at Tmax (1 hour), had declined to 0.023 to 0.083% of the dose by 72 hours after dosing. Mean percent of dose recovered at Tmax, Tmax+5h, and terminal sacrifice in tissues of male and female rats following a single 25 or 500 mg/kg bw single oral dose are presented in Tables 7-8.

Table 7. Percent of dose recovered at Tmax, Tmax+5h, and terminal sacrifice in tissues of male rats following a single 25 or 500 mg/kg bw single oral dose of ^{14}C-DPX-MAT28

Male	25 mg/kg bw			500 mg/kg bw		
	Tmax (1h)	Tmax+5 (6 h)	Terminal (72 h)	Tmax (1h)	Tmax+5 (6 h)	Terminal (72 h)
Sample	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose
carcass[a]	3.458 ± 1.065	5.167 ± 5.412	0.067 ± 0.028	4.154 ± 7.765	0.276 ± 0.050	0.058 ± 0.028
skin[b]	1.306 ± 0.552	0.073 ± 0.010	0.043 ± N/A.	0.780 ± 0.203	0.057 ± 0.013	N.A. ± N.A
whole blood[b]	1.020 ± 0.409	0.039 ± 0.012	N.A. ± N.A.	0.587 ± 0.102	0.029 ± 0.004	N.A. ± N.A.
bone marrow[b]	0.072 ± 0.017	0.053 ± N.A.	N.A. ± N.A.	0.042 ± 0.006	0.007 ± N.A.	N.A. ± N.A.
brain	0.004 ± 0.001	0.0008 ± 0.0001	N.A. ± N.A.	0.0024 ± 0.0004	0.0007 ± 0.0001	N.A. ± N.A.
fat[b]	0.238 ± 0.171	0.025 ± 0.011	N.A. ± N.A.	0.375 ± 0.519	0.023 ±0.007	N.A. ± N.A.
heart	0.026 ± 0.015	0.0012 ± 0.0002	N.A. ± N.A.	0.015 ± 0.002	0.0009 ± 0.0002	N.A. ± N.A.
lungs	0.036 ± 0.022	0.0019 ± 0.0004	N.A. ± N.A.	0.024 ± 0.008	0.0016 ± 0.0009	N.A. ± N.A.
spleen	0.006 ± 0.004	0.0006 ± 0.0001	N.A. ± N.A.	0.038 ± 0.062	0.0004 ± 0.0001	N.A. ± N.A.
liver	0.750 ± 0.392	0.038 ± 0.010	N.A. ± N.A.	0.392 ± 0.047	0.030 ± 0.007	N.A. ± N.A.
kidney	0.894 ± 0.334	0.025 ± 0.009	0.000 ± N.A.	0.562 ± 0.318	0.030 ± 0.019	0.0002 ± 0.0001
G.I. tract	13.083 ± 3.283	1.363 ± 0.110	0.001 ± N.A.	11.913 ± 2.420	1.753 ± 0.667	0.0016 ± 0.0002
G.I. contents	58.039 ± 5.571	46.766 ± 10.688	0.013 ±0.008	63.535 ± 5.997	67.032 ± 6.272	0.040 ± 0.018
pituitary	0.0004 ± 0.0003	N.A. ± N.A.	N.A. ± N.A.	0.002 ± 0.002	N.A. ± N.A.	N.A. ± N.A.
thyroid	0.0006 ± 0.0002	N.A. ± N.A.	N.A. ± N.A.	0.002 ± 0.001	0.0003 ± N.A.	N.A. ± N.A.
thymus	0.006 ± 0.003	0.0004 ± 0.0001	N.A. ± N.A.	0.004 ± 0.002	0.0004 ± 0.0001	N.A. ± N.A.
ovaries	--	--	--	--	--	--
testes	0.025 ± 0.009	0.0031 ± 0.0009	N.A. ± N.A.	0.031 ± 0.028	0.0020 ± 0.0006	N.A. ± N.A.
pancreas	0.023 ± 0.016	0.0014 ± 0.0006	N.A. ± N.A.	0.035 ± 0.018	0.0034 ± 0.0025	N.A. ± N.A.
adrenals	0.001 ± 0.001	0.0001 ± N.A.	N.A. ± N.A.	0.019 ± 0.021	0.0001 ± N.A.	N.A. ± N.A.
uterus	--	--	--	--	--	--
muscle[b]	1.189 ± 0.575	0.098 ± 0.048	N.A. ± N.A.	2.008 ± 2.099	0.057 ± 0.009	N.A. ± N.A.
bladder	0.235 ± 0.318	0.030 ± 0.023	N.A. ± N.A.	0.126 ± 0.162	0.003 ± 0.002	N.A. ± N.A.
bone[b]	0.088 ± 0.019	0.008 ± 0.001	N.A. ± N.A.	0.073 ± 0.022	0.018 ± 0.024	N.A. ± N.A.
Total	77.043 ± 1.827	48.500 ± 10.832	0.034 ± 0.027	80.567 ± 8.825	69.042 ± 6.094	0.042 ± 0.018

[a] Percent of dose recovered in carcass is not included in total.

[b] Percentages for tissues collected as partial samples were adjusted to estimate recovery in the full tissue weight as percent of terminal body weight: skin (19%), whole blood (7.4%), bone marrow (2.3%), fat (7.0%), muscle (40.4%), and bone (5%). (Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., and Beliles, R.P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health 13(4), 407-484).

Source: Tables 6 and 8, p. 50 and 52 of study report.

Table 8. Percent of Dose recovered at Tmax, Tmax+5h, and terminal sacrifice in tissues of female rats following a single 25 or 500 mg/kg bw single oral dose of ^{14}C-DPX-MAT28

Female	25 mg/kg bw			500 mg/kg bw		
	Tmax (1h)	Tmax+5 (6 h)	Terminal (72 h)	Tmax (1h)	Tmax+5 (6 h)	Terminal (72 h)
Sample	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose	Mean ± SD % of dose
carcass[a]	3.187 ± 0.590	0.354 ± 0.226	0.200 ± 0.109	2.462 ± 0.691	0.427 ± 0.241	0.101 ± 0.104
skin[b]	1.368 ± 0.278	0.060 ± 0.010	N.A. ± N.A.	0.695 ± 0.072	0.047 ± 0.010	N.A. ± N.A.
whole blood[b]	0.954 ± 0.297	0.034 ± 0.011	N.A. ± N.A.	0.492 ± 0.060	0.029 ± 0.007	N.A. ± N.A.
bone marrow[b]	0.076 ± 0.015	0.011 ± N.A.	N.A. ± N.A.	0.039 ± 0.007	0.005 ± N.A.	N.A. ± N.A.
brain	0.005 ± 0.002	0.001 ± 0.001	N.A. ± N.A.	0.0027 ± 0.0002	0.0008 ± 0.0001	N.A. ± N.A.
fat[b]	0.210 ± 0.072	0.019 ± 0.002	N.A. ± N.A.	0.106 ± 0.024	0.017 ± 0.002	N.A. ± N.A.
heart	0.021 ± 0.005	0.001 ± 0.000	N.A. ± N.A.	0.0108 ± 0.0012	0.0009 ± 0.0002	N.A. ± N.A.
lungs	0.038 ± 0.013	0.002 ± 0.001	N.A. ± N.A.	0.0232 ± 0.0093	0.0015 ± 0.0002	N.A. ± N.A.
spleen	0.006 ± 0.001	0.001 ± 0.001	N.A. ± N.A.	0.0031 ± 0.0007	0.0003 ± 0.0000	N.A. ± N.A.
liver	0.517 ± 0.118	0.023 ± 0.010	N.A. ± N.A.	0.264 ± 0.041	0.018 ± 0.004	N.A. ± N.A.
kidney	1.072 ± 0.277	0.051 ± 0.037	N.A. ± N.A.	0.431 ± 0.097	0.024 ± 0.010	0.0003 ± N.A.
G.I. tract	9.544 ± 6.923	1.428 ± 0.496	0.001 ± N.A.	10.847 ± 3.341	3.928 ± 3.125	0.0029 ± 0.0026
G.I. contents	51.504 ± 4.976	41.749 ± 7.510	0.022 ± 0.025	64.058 ± 12.863	56.035 ± 2.715	0.080 ± 0.066
pituitary	0.001 ± 0.000	N.A. ± N.A.	N.A. ± N.A.	0.0003 ± 0.0001	N.A. ± N.A.	N.A. ± N.A.
thyroid	0.001 ± 0.000	N.A. ± N.A.	N.A. ± N.A.	0.0010 ± 0.0006	N.A. ± N.A.	N.A. ± N.A.
thymus	0.007 ± 0.002	0.000 ± 0.000	N.A. ± N.A.	0.0039 ± 0.0013	0.0003 ± 0.0001	N.A. ± N.A.
ovaries	0.006 ± 0.003	0.000 ± 0.000	N.A. ± N.A.	0.0029 ± 0.0016	0.0003 ± 0.0002	N.A. ± N.A.
testes	--	--	--	--	--	--
pancreas	0.020 ± 0.007	0.001 ± 0.001	N.A. ± N.A.	0.0187 ± 0.0137	0.0007 ± 0.0002	N.A. ± N.A.
adrenals	0.005 ± 0.005	0.000 ± N.A.	N.A. ± N.A.	0.0011 ± 0.0003	0.0001 ± N.A.	N.A. ± N.A.
uterus	0.018 ± 0.007	0.0008 ± 0.0004	N.A. ± N.A.	0.0182 ± 0.0146	0.0010 ± 0.0010	N.A. ± N.A.
muscle[b]	0.993 ± 0.257	0.067 ± 0.011	N.A. ± N.A.	0.385 ± 0.092	0.052± 0.006	N.A. ± N.A.
bladder	0.124 ± 0.057	0.004 ± 0.003	N.A. ± N.A.	0.138 ± 0.141	0.003 ± 0.002	N.A. ± N.A.
bone[b]	0.104 ± 0.025	0.007 ± 0.001	N.A. ± N.A.	0.049 ± 0.012	0.006 ± 0.001	N.A. ± N.A.
Total	66.594 ± 5.003	43.453 ± 8.030	0.023 ± 0.026	77.591 ± 9.556	60.168 ± 1.977	0.083 ± 0.069

[a] Percent of dose recovered in carcass is not included in total.

[b] Percentages for tissues collected as partial samples were adjusted to estimate recovery in the full tissue weight as percent of terminal body weight: skin (19%), whole blood (7.4%), bone marrow (2.3%), fat (7.0%), muscle (40.4%), and bone (5%). (Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., and Beliles, R.P. (1997). Physiological parameter values for physiologically based pharmacokinetic models. Toxicology and Industrial Health 13(4), 407-484).

Source: Tables 7 and 9, p. 51 and 53 of study report.

4. **Multiple Dose Administration:** The overall mean material balance after multiple dose administration was 97.0% and 98.0% in male and female rats, respectively. After multiple oral dose administration (25 mg/kg bw/day x 14 days), the majority of the dose was recovered in urine and feces (Figure 9). The mean total percent of excretion was 38.8% and 36.6% in the urine and 55.3% and 57.3% in the feces of male and female rats, respectively. The overall pattern of excretion during and after multiple dosing appeared to be the most similar to the data from the single high (500 mg/kg bw) dose group which had mean recovery values of 40.0% and 44.1% in urine and 54.8% and 51.1% in feces of male and female rats, respectively. The majority of the remainder of the mean recovery was accounted for in cage wash (2.62 and 4.04%) and residual feed (0.253% and 0.146%) for male and female rats, respectively. Like the single dose data, the mean percent of the dose recovered in tissues and carcass was minor and accounted for 0.03% of the cumulative dose for either sex.

The tissue distribution of ^{14}C-DPX-MAT28 was evaluated extensively with the objective of identifying changes caused by multiple vs. single dose administration. During dosing, the tissue collection times were designed to occur 6 hours after the last respective dose and thus coincided for comparison with the initial 6-hour sample time from the single low (25 mg/kg bw) dose group. Selected tissues (plasma, rbc, whole blood, fat, liver, kidney and muscle) were collected at multiple time points for male rats. Fewer time points were used for female rats as summarized in the experimental design above (see Materials and Methods) based on the observation that plasma kinetic data were similar between sexes.(1) The concentration time course of ^{14}C residues showed a rapid increase to apparent steady state by 3 days of repeated oral dose administration (the first day of dosing was designated as Day 0). The mean concentrations were very similar at the next 2 time points (Days 6.25 and 10.25) but showed a jump at Day 13.25. The reason for the apparent increase was a change in the dose concentration used for the last day of dosing. The dose level on the last day of dosing was approximately 5 dose units higher than on the previous days. This concentration increase in the tissues was a dose dependent event and not because of increasing accumulation of ^{14}C residues. After this last dose, the concentrations in the selected tissues of male rats showed a rapid decline. One minor exception was the fat which exhibited similar mean concentrations at the last 4 sample times of approximately 0.1 (i.e. ^{14}C-equivalents/g, while the other tissues such as liver, kidney and muscle concentrations declined to mean values of 0.02, 0.03 and 0.015 ng equivalents/g respectively by 72 hours after the last dose. Although this observation in fat suggests minor retention of 14C residues, it is important to note that the percentage in any of the selected tissues including fat were substantially less than 0.01% of the cumulative administered dose. On Day 15 (48 hours after the last dose), tissue:plasma concentration ratios were the highest in fat (7:1) followed by kidney (4:1), liver (2.2:1) and muscle (1.1:1). Concentration ratios for whole blood and red blood cells were <1. The absence of quantifiable ^{14}C residues in plasma precluded calculation of ratios at Day 16 (72 hours after the last dose.

Fewer representative sample points were used for female than for male rats for the selected tissue samples. The tissue distribution data for female rats were very similar to those obtained for male rats for percent of dose, concentration, and tissue:plasma concentration ratios. None of the selected tissues in male or female rats had tissue residues that were greater than 0.1% of the total dose at any of the sample times during or following multiple dose administration.

In addition to the evaluation of tissue distribution in selected tissues, a comprehensive evaluation was conducted in the full list of tissues collected from male and female rats at 6 and 72 hours after the last multiple dose. The samples with the highest concentration of ^{14}C residues and percentage of the dose 6 hours after the last dose were the gastrointestinal (GI) tract and its contents. The percentage of the dose was considerably greater in the GI contents than the GI tract tissue, containing approximately 7.1 and 0.2% of the total dose, respectively. Similar percentages of ^{14}C residues were evident in male and female rats. By 72 hours after the last dose, these declined to mean values of 0.003% for the GI contents and 0.0002% for the GI tissue; the mean percentages were similar in male and female rats. Total tissue burdens (percentages) were similar between sexes and declined from mean values of 7.3-7.4% at 6 hours to 0.008-0.01% by 72 hours after administration of the last multiple oral dose. The final 72-hour total tissue percentages (0.008-0.01%) after multiple dosing were lower but

qualitatively similar to those measured (0.023%-0.083%) after single dose administration. Overall, the pattern of tissue distribution for percent, concentration and tissue:plasma concentration ratios for multiple dose administration indicated no potential for accumulation, and the patter of distribution was similar to that observed after single dose administration. Material balance and mean percent of dose recovered for multiple dosing are presented in Tables 9-10.

Table 9. Material balance for the multiple dosing experiment with ^{14}C-DPX-MAT28 at Day 16

	25 mg/kg bw	
	Male	Female
Sample	Mean ± SD (% of dose)	Mean ± SD (% of dose)
Urine	38.85 ± 3.94	36.57 ± 2.38
Feces	55.26 ± 3.50	57.25 ± 2.87
Cage Wash	2.62 ± 0.92	4.04 ± 0.70
Residual feed	0.253 ± 0.238	0.146 ± 0.061
Tissue + carcass	0.0300 ± 0.0128	0.0263 ± 0.0061
Material Balance	97.01 ± 1.92	98.03 ± 0.23

Source: Table 16, p. 60 of study report.

Table 10. Percent recovery of ^{14}C residues and tissue:plasma ratio at Day 13.25 and 16 after 14-day oral gavage administration of 25 mg/kg bw ^{14}C-DPX-MAT28

	Male				Female			
	Day 13.25 (6 h after last dose)	Day 16 (72 h after last dose)	Tissue:Plasma Ratio		Day 13.25 (6 h after last dose)	Day 16 (72 h after last dose)	Tissue:Plasma Ratio	
Sample	Mean ± SD % of dose	Mean ± SD % of dose	Day 13.25	Day 16	Mean ± SD % of dose	Day 13.25	Day 13.25	Day 16
carcass[a]	0.13774 ± 0.01465	0.02629 ± 0.01206	1.03	N.A.	0.13374 ± 0.01184	0.02298 ± 0.00787	1.98	N.A.
skin[b]	0.01369 ± 0.00231	0.00270 ± 0.00042	0.42	N.A.	0.01021 ± 0.00170	0.00239 ± 0.00038	0.60	N.A.
Plasma	---	---	1.00	N.A.	---	---	1.00	N.A.
rbc	---	---	0.38	N.A.	---	---	0.40	N.A.
whole blood[b]	0.000806±0.00106	0.00020 ± N.A.	0.63	N.A.	0.00432 ± 0.00188	0.00020 ± N.A.	0.66	N.A.
bone marrow[b]	N.A. ± N.A.	0.00103 ± N.A.	N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A.
brain	0.00009 ± 0.00001	0.00002 ± N.A.	0.09	N.A.	0.00012 ± 0.00001	0.00003 ± N.A.	0.16	N.A.
fat[b]	0.00590 ± 0.00234	0.00245 ± 0.00053	0.49	N.A.	0.00303 ± 0.00008	0.00221 ± 0.00012	0.48	N.A.
heart	0.00016 ± 0.00003	0.00002 ± N.A.	0.29	N.A.	0.00012 ± 0.00003	N.A. ± N.A.	0.40	N.A.
lungs	0.0003 ± 0.00017	0.00003 ± 0.00001	0.46	N.A.	0.00034 ± 0.00024	0.00003 ± N.A.	0.77	N.A.
spleen	0.00010 ± 0.00007	0.00001 ± N.A.	0.29	N.A.	0.00005 ± 0.00001	N.A. ± N.A.	0.26	N.A.
liver	0.01036 ± 0.00422	0.00031 ± 0.00001	1.46	N.A.	0.00280 ± 0.00088	0.00020 ± 0.00002	0.79	N.A.
kidney	0.00532 ± 0.00056	0.00007 ± 0.00001	3.95	N.A.	0.00329 ± 0.00228	0.00012 ± 0.00011	4.91	N.A.
G.I. tract	0.21929 ± 0.04131	0.00022 ± 0.0001	78.48	N.A.	0.16495 ± 0.07986	0.00018 ± 0.00004	88.80	N.A.
G.I. contents	7.14252 ± 0.56604	0.00261 ± 0.00008	738.34	N.A.	7.10525 ± 0.64665	0.00255 ± 0.00182	1024.58	N.A.
pituitary	N.A. ± N.A.	0.00001 ± N.A.	N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A.
thyroid	N.A. ± N.A.	0.00001 ± N.A.	N.A.	N.A.	N.A. ± N.A.	N.A. ± N.A.	N.A.	N.A.
thymus	0.00006 ± 0.00002	0.00002 ± N.A.	0.20	N.A.	0.00006 ± 0.00001	N.A. ± N.A.	0.27	N.A.
ovaries	---	---	---	N.A.	N.A. ± N.A	N.A. ± N.A.	N.A.	N.A.
testes	0.00112 ± 0.00117	0.00004 ± N.A.	0.82	N.A.	---	---	---	N.A.
pancreas	0.00030 ± 0.00022	0.00002 ± N.A.	0.84	N.A.	0.00013 ± 0.00009	N.A. ± N.A.	0.45	N.A.
adrenals	0.00003 ± N.A.	0.00001 ± N.A.	1.26	N.A.	N.A. ± N.A	N.A. ± N.A.	N.A.	N.A.
uterus	--	--		N.A.	0.00009 ± 0.00003	N.A. ± N.A.	0.57	N.A.
muscle[b]	0.03461 ± 0.02867	0.00202 ± N.A.	0.49	N.A.	0.00854 ± 0.00214	N.A. ± N.A.	0.24	N.A.
bladder	0.00336 ± 0.00347	0.00001 ± N.A.	52.63	N.A.	0.00048 ± 0.00056	N.A. ± N.A.	17.22	N.A.
bone[b]	0.00126 ± 0.00049	0.00034 ± N.A.	0.15	N.A.	0.00068 ± 0.00010	N.A. ± N.A.	0.15	N.A.
Total	7.446 ± 0.590	0.010 ± 0.002	---	N.A.	7.304 ± 0.726	0.008 ± 0.002	---	N.A.

Source: Extracted from Tables 21-22, pp. 67-70 of study report.

B. METABOLITE CHARACTERIZATION STUDIES:

No metabolites were found for DPX-MAT28. Parent chemical and potential metabolites were quantified by collecting fractions of HPLC effluent from all runs onto 96-well scintillation plates, drying the plates, and counting of radioactivity. Identification and confirmation of parent and potential metabolites was conducted by splitting about 1% of the HPLC effluent from the same analysis directly to the mass spectrometer. Identified components (e.g. DPX-MAT28) were confirmed by MS full scan and daughter ion scans of each component with the respective reference standard.

Urine

DPX-MAT28 was the sole identified component in urine and represented 47.35% and 56.06% of the single low (25 mg/kg bw) dose and 39.80% and 43.61% of the high (500 mg/kg bw) dose for male and female rats, respectively over 24 hours. After multiple, low dose administration, DPX-MAT28 in urine of male rats was 38.29%, 39.20% and 38.77% at the 0-24, 120-144, and 264-288 hour collection intervals, respectively. In the urine of female rats, the values were 41.48%, 37.69%, and 31.99% at the same collection intervals. These data indicated an overall range of 32-56% of administered DPX-MAT28 excreted in urine after single low, high or multiple low dose administration. The magnitude of excretion appeared to decline with increasing dose after single oral administration. The percent of excretion during multiple dose administration was most similar to that observed after single high dose administration.

Feces

DPX-MAT28 was the sole identified component in feces and represented 47.43% and 39.24% of the single low (25 mg/kg bw) dose and 51.71% and 55.94% of the high (500 mg/kg bw) dose for male and female rats, respectively. After multiple dose administration, DPX-MAT28 in feces of male rats was 45.06%, 53.75% and 54.53% at the 0-24, 120-144, and 264-288 hour collection intervals, respectively. In the feces of female rats, the values were 43.86%, 54.03%, and 55.20% at the same collection intervals. These data indicated an overall range of 39-56% of administered DPX-MAT28 excreted in feces after single low, high or multiple low dose administration. The magnitude of excretion showed a clear increase with increasing dose after single oral administration. The percent of excretion during multiple dose administration was most similar to that observed after single high dose administration.

Bile

At a much lower percentage of the total dose, DPX-MAT28 was the sole identified component in bile and represented 0.213% and 0.093% of the single low (25 mg/kg bw) dose and 0.153% and 0.100% of the high (500 mg/kg bw) dose for male and female rats, respectively. These data indicated an overall range of 0.093-0.213% of administered DPX-MAT28 excreted in bile after single low and high dose administration. The magnitude of excretion was essentially the same at both dose levels.

Reference Standard

No reference standard was detected in excreta. No match was found for the reference standard IN-LXT69 in any urine, feces or bile samples from the current study.

Proposed Metabolic Pathway

DPX-MAT28 when administered as a single or repeated dose, is excreted intact in the urine, feces, and bile.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** Material balance was investigated after low (25 mg/kg bw) and high (500 mg/kg bw) single oral gavage dose administration, and after low multiple (25 mg/kg bw/day x 14 day) oral gavage dose administration. Recoveries were excellent with mean values that ranged from 97.4% to 99.4% for all experimental groups. No ^{14}C residues were detected in exhaled breath as $^{14}CO_2$ or ^{14}C-volatiles after single low (25 mg/kg bw) dose administration. By 72 hours after single or multiple dose administration, the body burden (tissue + carcass) was very low and accounted for 0.030% to 0.222% of the dose. At both single dose levels, the majority of the dose was excreted between the urine and feces in a roughly proportional manner within 24 hours after dose administration. By 72 hours, the mean percent of the dose in urine ranged from 40% to 56.5% with a slight reduction in total percentage of dose excreted at the high (500 mg/kg bw) dose relative to the low (25 mg/kg bw) dose. The mean excretion in feces ranged from 39.5% to 54.8% of the dose with a slightly higher percentage excreted at the high (500 mg/kg bw) than low (25 mg/kg bw) dose. This difference most likely reflects a slight reduction in total absorption at the high dose. Total excretion in urine and feces after multiple dose administration was most similar to the single high dose group and did not change substantially between the first and last day of dose administration. In all cases, a sex difference in the excretion pattern was not evident.

The percent of absorbed dose was measured in bile duct cannulated rats for up to 48 hours after single low (25 mg/kg bw) and high (500 mg/kg bw) dose administration. The majority of the absorbed dose was excreted in the urine at both dose levels (22.2% to 34.5%). Only a small percentage was recovered in bile (0.13% to 0.25%), with the sum of the dose in urine + bile + carcass (less the GI tract contents) accounting for 22.4% to 34.9% of the dose. Sex or dose dependent differences were not evident. The estimated absorption from the bile duct cannulated rats was low compared with the cumulative 48-hour excretion in urine of non-bile duct cannulated rats from the material balance experiment. At 48-hours in these non-cannulated rats, the urine contained 39.9% to 56.2% of the administered low and high doses. In the bile duct cannulated rats, the majority of the dose was un-absorbed and excreted in the feces (57.9% to 68.5%), which in comparison to non-cannulated rats suggested that conditions within the biliary elimination experiment reduced systemic absorption from the gastrointestinal tract. In this case, the urinary excretion + tissue + carcass from the single and multiple dose material balance experiments appears to provide a better estimate of the absorbed dose, accounting for 37% to 57% by 72 hours after dose administration.

Tissue distribution experiments demonstrated systemic uptake of ^{14}C-DPX-MAT28 based on quantifiable residues shortly (1 and 6 hours) after single dose administration. For example, the mean total body burdens, which ranged from 67% to 81% of the dose at Tmax (1 hour), had declined to 0.023% to 0.083% of the dose by 72 hours after single dose administration. After multiple dose administration, total body burdens (percentages) were similar between sexes and declined from mean values of 7.3-7.4% at 6 hours to 0.008-0.01% by 72 hours after administration of the last dose. Based on the extensive analysis after single or multiple dose administration, the percent of dose, concentration, and tissue:plasma concentration ratio data indicated no potential for accumulation of ^{14}C-DPX-MAT28.

The analysis of urine, feces and bile for potential metabolites clearly showed that ^{14}C-DPX-MAT28 was the sole component in excreta. No metabolism was evident.

B. REVIEWER COMMENTS:

A previous study (MRID 47560023) characterized the plasma pharmacokinetics of DPX-MAT28 in male and female rats after administration of a single low or high dose and provided a pilot material mass balance.

In metabolism studies (MRID 48333610), [pyrimidine-2-^{14}C]-aminocyclopyrachlor (^{14}C -DPX-MAT28) (99.5% radiochemical purity) in 0.5% methylcellulose was administered by a oral gavage dose to male and female Sprague-Dawley Crl:CD(SD) rats (4/sex/dose). Control animals (1/sex/dose) were administered 0.5% methylcellulose. Different study designs were employed to investigate metabolism and disposition.

In the first study, ^{14}C -DPX-MAT28 was administered at a single dose of 25 or 500 mg/kg bw. Total recovery and recovery in urine, feces, and as exhaled CO_2 were monitored for 72 hours. Total recovery was excellent at ≥97.4%. Negligible amounts were exhaled as CO_2. Recoveries in urine and feces were similar for both sexes and doses (with feces being slightly higher) and was near-complete by 72 hours. Recoveries in urine peaked at 6 hours, whereas recoveries in feces peaked at 12-24 hours. At 72 hours, percent recoveries in urine were 47.74 ± 3.75 and 40.04 ± 5.14 for males at 25 and 500 mg/kg bw. For females, these values were 56.51 ± 14.55 and 44.08 ± 4.78, respectively. Percent recoveries in feces at 72 hours were 47.97 ± 3.32 and 54.76 ± 5.14 for males at 25 and 500 mg/kg bw. For females, these values were 39.48 ± 13.42 and 51.08 ± 3.33and 56.51 ± 14.55, respectively.

In the second study, biliary elimination was examined by administration of a single dose of 25 or 500 mg/kg bw in bile duct-cannulated rats for 48 hours. Total recovery and recovery in urine, feces, and bile were monitored for 48 hours. Total recovery was excellent at ≥95.1%. Maximum recoveries and times of peak recoveries in urine and feces were similar to uncannulated rats. However, a larger fraction of radioactivity was increased in the feces than the urine. At 48 hours, percent recoveries in feces were 62.06 ± 3.41 and 57.86 ± 7.77 for males at 25 and 500 mg/kg bw and 68.47 ± 6.59 and 60.68 ± 4.87, respectively, for females. In urine at 48 hours, percent recoveries were 34.52 ± 3.57 and 30.06 ± 7.89 at 25 and 500 mg/kg bw for males and 22.18 ± 2.13 and 32.24 ± 5.60, respectively for females. Maximum excretion in the bile for both sexes occurred by 6 hours. However, total excretion in the bile within 48 hours was low at less than 0.3% for both sexes at both doses. Together, this data indicate a minor role for enterohepatic circulation in the metabolism of ^{14}C -DPX-MAT28.

The tissue distribution of radiolabel was observed at T_{MAX} (1 hour), T_{MAX} + 5 hours, and 72 hours following a single oral (gavage) dose of 25 or 500 mg/kg bw in males/females. The majority of the dose was found in the GI contents (46.7-67.0%) and GI tract (9.54-13.08%) in both sexes at both doses by T_{MAX} + 5 hours without an apparent dose-related increase. Radioactivity was largely absent in the GI contents/GI tract by 72 hours (<<1%). The highest levels or radiolabel for both sexes and doses were found in the carcass (up to about 5%), followed by the muscle (up to

2%), skin (up to 1.4%), and blood (up to 1%). Again, there was no apparent dose-related increase in tissue levels. However, levels in all of these tissues declined to very low levels (<0.5%) by 72 hours. The apparent lack of dose-related increase in the tissue content of radiolabel suggested saturation of uptake.

Tissue distribution after repeated dosing the 25 mg/kg bw/day for 14 days was also examined. On Day 13 at 6 hours following dose administration, Levels were again highest in the GI tract (0.16-0.22%) and GI contents in males/females (7.11-1.14%), but were reduced in magnitude compared to 6 hours following single-dose administration of 25 mg/kg in the GI tract (1.36-1.42%) and GI contents (41.7% and 46.77%) in both sexes. Tissue distribution was similar with carcass > skin > blood, with all levels less than 0.5% within 6 hours of dosing and less than 0.05% by 72 hours.

The analysis of urine, feces and bile for potential metabolites clearly showed that ^{14}C-DPX-MAT28 was the sole ^{14}C component in excreta. No metabolism was evident.

This metabolism study in the rat is classified **acceptable/guideline** and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485 (§85-1)] in rats.

C. **STUDY DEFICIENCIES:** None.

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R195091

Chemical Name: Aminocyclopyrachlor
Aminocyclopyrachlor methyl ester

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